A Handbook on

Disorders of Sex Development

Dr. V.R. Ravikumar
Coimbatore

Printed on behalf of Coimbatore Association of Paediatric Surgeons

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Preface

Disorders of sex development (DSD) is an enigmatic condition involving various complex issues in the management of children. There are variations in the management of DSD, not only between different continents and cultures, but also within countries and to a certain extent among centers in the same country. These are rare anomalies and need long term follow up till they attain adulthood and married life.

Management options are highly controversial since consent from the child involved cannot be obtained directly and to wait till the child attains adolescence to give informed consent which is not practicable in certain situations. The experience of most doctors involved in gender assignment indicates that many, if not most, parents would find it extremely difficult to contemplate rearing their child without cosmetic surgery.

Random articles on anecdotal evidence of dissatisfaction among adult patients who underwent childhood surgery, make clinicians and parents face huge dilemmas since there are no clinical trials providing data on outcomes for cosmetic, gender, social or sexual function after the surgery.

A single individual having a large series is rare except in major teaching institutions in our country. There has been progress in diagnostic methods, surgical techniques and long term psychosocial, and sexual aspects of women who had surgery in childhood. The need to involve different specialties in decision making is emphasised. Apart from making a proper diagnosis, the need to provide these children with optimal medical and surgical treatment and appropriate counseling is emphasised in the respective chapters. With every

chapter, there are case histories with long term follow up. In 46 XX DSD, the main emphasis is on congenital adrenal hyperplasia since it is the commonest cause. Amongst the various causes of 46XY DSD with under virilization of external genitalia, 5 alpha reductase deficiency, and complete and partial androgen insensitivity are dealt with in detail while other conditions are discussed in the differential diagnosis. Management issues in mixed gonadal dysgenesis, Ovo testicular DSD and Persistent Mullerian Duct Syndrome are outlined with clinical case studies.

The book is written to make the complex subject easy to understand and apply it in clinical practice.

I thank Professor Jayant Radhakrishnan, Emeritus Professor of Surgery and Paediatric Urology from the University of Illinois, Chicago for writing the foreword for the booklet.

I thank Professor Minu Bajpai who has taken great pains to go through the whole book and has written a foreword outlining the salient features of each chapter.

I thank Dr. Ahila Ayyavoo Paediatric Endocrinologist GKNM Hospital Coimbatore for providing case reports. Thanks to Dr. Muthulingam & Dr. Madhu for complete proof reading and correction of the manuscript. Thanks to Dr. Rajeeve Redkar Chief Editor IAPS textbook of Paediatric Surgery for permitting to publish photographs of my article from the textbook.

I thank Prof. Sudipta Sen for the line diagram and the photograph on surgical steps of clitoroplasty. Thanks to Dr. Yogendra Singhvi for his case report on Persistent Mullerian Duct Syndrome.

Thanks to Mr. Rajendran of Raasi Enterprises Coimbatore who has done a commendable job in bringing out the booklet in nice format.

Dr. V.R. Ravikumar
Coimbatore

Foreword

In this handbook on Disorders of Sex Development Dr. Ravikumar has achieved something that is rarely possible. He has detailed all the intricate details that a person who takes care of these patients and postgraduates interested in the subject should know. On the other hand, a person who just wishes to know what to do and more importantly, what not to do in a given case only has to read the case reports and key points in each chapter to care for the patient appropriately.

I believe this handbook would be an excellent reference source in all medical college libraries and for anyone who cares for these patients.

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Foreword

There are misunderstandings attached with DSD due to ignorance. These conditions are caused by genetic and endocrine imbalances in foetal life and children are born with genital appearances which do not conform to clearly male or female genitals. The infant may be often rejected by the parents and generally discriminated by the society.

Adequate literature is available on these conditions, but, the hand book written by Prof. V.R. Ravikumar is unique as it brings forward many years of the author's experience in treating these anomalies. With every chapter there are case histories & a narrative of the long term follow ups. The book begins with an introduction to the Indian scenario in disorders of sex development, nomenclature and initial clinical evaluation. At the end of each chapter key points have been listed.

Congenital Adrenal Hyperplasia (CAH) is the most common DSD in the west but second most common reported in India. The book discusses the various presentations of CAH in the classical and non-classical forms, steps to detect carrier state, imaging & clinical practice guidelines. There are vivid descriptions on CAH & pregnancy, sports & gender as related to CAH, CAH child reared as boy & the late management issues. The subject of prenatal therapy is also dwelt upon.

In 46XY DSD, the description on 5 ά reductase deficiency has been deeply discussed with the author's personal experience on the subject easily visible. In the section on Mixed Gonadal Dysgenesis the intriguing subject of gender assignment & its management reflects the author's fine judgmental skills. In Ovotesticular, there have been

several reports of successful pregnancies and the same has been lucidly documented in the author's own clinical experience.

Persistent Mullerian duct syndrome is a difficult diagnosis to make when children with bilateral impalpable testes are managed without a high index of suspicion for this condition. This section highlights in detail the precautions to be taken during surgery for PMDS. The book ends with an overview on how best to approach the parents & the families with a DSD child.

Overall, the book has been written in an easily readable form & would serve as a guide for those managing DSD in its varied clinical presentations. The author needs to be complimented for making a 'deep dive' into long years of his experience and bringing to the readership the meticulously documented cases on all fronts, i.e., the clinical presentations, diagnostic findings, medical & surgical interventions & at same time giving outstanding insights into DSD.

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Chapter 1

Introduction to the Indian Scenario of Disorders of Sexual Development, Nomenclature and Initial Clinical Evaluation

When a child is born the first question asked by the parents and relatives is "is it a boy or girl?" This is more so in India where the disclosure of the sex by ultrasound of the newborn is not legal. The newborn infant with ambiguous external genitalia often comes as a surprise for the doctors as well as the parents. This is a social and in some cases endocrine emergency as in congenital adrenal hyperplasia. It should be emphasized that gender assignment must be done only after a thorough evaluation of the newborn by an experienced multi disciplinary team. Open communication with patients and families are essential and participation in decision making is encouraged. With the current availability of laboratory investigations, it is possible to arrive at a reasonable diagnosis of genetic sex within fortyeight hours, but the problem arises in assigning the sex of rearing based on the clinical appearance of external genitalia. Confirming a cause for the newborn with ambiguous genitalia and devising a management plan is one of the most challenging clinical conditions for the paediatric surgeon and the optimum management should involve paediatric endocrinologist, paediatrician, paediatric surgeon and psychiatric counsellor.

It is important to diagnose disorders of sex development (DSD) correctly as soon as possible to counsel the parents appropriately. Establishing a precise diagnosis in DSD is just as important as in other chronic medical conditions with lifelong consequences. The initial contact with the parents of a child with a DSD is important, as first

impressions from these encounters often persist. A key point to emphasize to the parents is that the DSD child has the potential to become a well-adjusted, functional member of society. Time has to be spent with parents explaining the sexual development with pictorial and web-based information of similar cases and will give confidence in arriving at the proper management of these children.

Problems in Indian scenario in a child with disorders of sexual differences

Lack of basic understanding of the complexities associated with DSD and lack of knowledge in those involved soon after delivery of the child is the major problem. There is a lack of uniformity in decision making in rearing the child with ambiguity with correct medical and surgical treatment. Any decision made by parents and surgeons in these may not be acceptable to the children as they grow up and can later turn into a legal issue after he/she grows up. This situation has not occurred in India so far since Indian children have complete faith and trust in their parents and the doctors. Even in older children with DSD, the decision regarding either continuation of rearing sex or change of sex is by the paternalistic decisions rather than the desires of the grown-up child. In older children, the gender of rearing would already have been established, and it is very difficult to change it. So, in many cases, the management has been decided by the parents according to the established gender and not based on what is best for the child and what the child wants.

Should we wait till the child is 18 years old to decide the sex of rearing in doubtful cases? Long wait till puberty for final sex preference is practically impossible in India. A child cannot say in the school that he or she will decide whether to be a male or female only after 18 years of age. It is preferable to give them a gender identity as early as possible even before they become conscious of their gender. In India, the decision to postpone on rearing sex is not acceptable to parents. When

in doubt male gender is preferred especially amongst the low socio-economic group, though it is changing slowly. It is considered that it is easier for an imperfect man to live than an imperfect female.

Definitive labelling of the child either as male or female is necessary for a number of social reasons in India. Mention of sex for admission to school or day-care is a must. There is a need to mention the definitive sex in the birth certificate. There is social stigma in the society and the existence of children with DSD is not widely known. There is a lack of awareness in the public and even in legal circles about the congenital condition and equate it with transgenders who are totally different. The visit of Hijras to the household where a baby is born for extortion especially in some states is a major problem. The choice of sex of rearing in the Indian scenario and in some Middle East countries, male is the preferred sex of rearing by many families. This may be detrimental in some situations, when a female sex may have been a better choice. Where the man is the traditional breadwinner, having a dominant role in institutional and social life, and the woman is the housewife and mother, a parental preference for the male gender is more important than the individual's sexual potential. With the improvement in economy, education and emancipation of women the trend is changing.

Genital surgery is now one of the most controversial interventions in disorders of sexual differentiation management. While genetically female children with ambiguous genitalia have less decision-making problems, genetically male children who are under virilized and reared as females face the maximum problems as they attain puberty and the present consensus is to rear them as male children.

Summarizing; gender assignment should be given to all newborns. Concerns of parents and families should be addressed and their participation in decision making is encouraged and confidentiality is maintained.

Nomenclature

Terms such as 'intersex','pseudohermaphroditism', 'hermaphroditism', Ambiguous genitalia and 'sex reversal' are no longer used and considered particularly controversial.

The term Disorders of Sex Development (DSD), as defined is a congenital discrepancy between external genitalia, gonadal and chromosomal sex, and has been proposed to replace the term 'intersex'. A classification is proposed in which DSDs associated with sex chromosome abnormalities (sex chromosome DSD) were separated from DSDs with a normal chromosome complement (46 XX DSD and 46 XY DSD). (Fig. 1)

The old terminology of ambiguous external genitalia, intersex and male and female pseudohermaphrodites are no longer used and the current classification is given below.

Previous	Revised
Intersex	Disorder of sex development
Male pseudohermaphrodite Undervirilization of an XY male	46, XY DSD
Female pseudohermaphrodite Overvirilization of an XX female	46, XX DSD
True hermaphrodite	Ovotesticular DSD
XX male or XX reversal	46,XX testicular DSD
XY sex reversal	46,XY complete gonadal dysgenesis

Fig. 1 - Revised Nomenclature (Lee et al)[2]

The DSD for better understanding has further been classified by the Chicago Consensus. (Fig. 2)

Sex Chromosome DSD	46 - XY DSD	46 - XX DSD
45, X (Turner syndrome and variants)	Disorder of gonadal (testicular) development	Disorder of gonadal (ovarian) development
47, XXY (Klinefelter syndrome and variants)	Complete gonadal dysgenesis (Swyer syndrome)	Ovotesticular DSD
45, X/46, XY (mixed gonadal - dysgenesis)	Partial gonadal dysgenesis	Testicular DSD (SRY+, dupSOx9)
46, XX/46, XY (chimeric, ovotesticular DSD)	Gonadal regression Ovotesticular DSD	Gonadal dysgenesis
	Disorders in androgen synthesis or action Androgen biosynthesis defect (17-hydroxysteroid dehydrogenase deficiency, 5α-reductase deficiency)	Androgen excess Fetal (21- or 11-hydroxylase deficiency)
	Defect in androgen action (CAIS, PAIS) LH receptor defects (Leydig cell hypoplasia)	Fetoplacental (aromatase, POR)
	Disorders of AMH and AMH receptor (persistent mullerian duct syndrome)	Maternal (luteoma, exogenous)
	Other (severe hypospadias, cloacal exstrophy)	Other (cloacal exstrophy, MURCS)

Fig. 2 - DSD Classification proposed by the Chicago Consensus

DSD, disorder of sex development; CAIS, complete androgen insensitivity syndrome; PAIS, partial androgen insensitivity syndrome; LH, luteinizing hormone; AMH, anti-mullerian hormone; POR, cytochrome P450 oxidoreductase; MURCS, mullerian duct aplasia; renal aplasia, and cervicothoracic somite dysplasia. (Lee PA, Houk CP, Ahmed SF, Hughes IA) [2]

Terminologies that are often confused

Gender identity refers to a person's self representation as male or female though some individuals may not identify exclusively with either. Gender role (sex-typical behaviours) describes the psychological characteristics that are sexually dimorphic within the general population, such as toy preferences, play and playmate preferences and physical aggression.

Sexual orientation refers to the direction of erotic interest (heterosexual, bisexual, homosexual) and includes behaviour, fantasies and attractions. Gender dysphoria denotes dissatisfaction with one's assigned gender and confused with DSD. In disorders of sexual differences, often the children are born with external genitalia malformation which gives the ambiguity to the sex and the term transgender is loosely applied to all these conditions even in legal circles.

Conditions and terminologies which are interchanged with DSD

Transgender refers to normal individuals whose gender expression that differs from their assigned sex. Transsexuals are those who desire medical assistance to transition from one sex to another. These are usually men who get castrated, have breast and vaginal reconstruction done and believe that he/ she is of a sex different from the biologic or assigned sex. Transvestite refers to individuals with sexual interest in cross-dressing, who habitually and voluntarily wear clothes of the opposite sex. LGBTQIA : It is an acronym for a group of people who want their identity recognized as a group of lesbians, gay, bisexual, transgender, queer and ally, intersex and asexual

Initial Clinical Evaluation

Family history of sudden-un explained deaths in siblings in a newborn with ambiguous genitalia should alert to the possibility of Congenital adrenal hyperplasia. A family history of similar problems in siblings or maternal aunts will indicate the autosomal recessive traits.

Maternal intake of hormones in the first trimester of pregnancy or history of ovarian tumour such as Arrhenoblastoma of ovary in the mother may be responsible for clitoromegaly.

Physical examination that suggests DSD include enlarged clitoris in an apparently female child with posterior labial fusion. This vary according to Prader's criteria and range from enlarged clitoris with normal vulval appearance to complete fusion of the labial folds up to the corona resembling distal hypospadias.

The newborn may present with micropenis, hypospadiac urethra with undescended testis with unfused labioscrotal folds in an apparent male child. If the gonads are palpable in the scrotum, it is testis. If only one gonad is palpable with external ambiguity it can be ovotestis.

If prenatal karyotyping has been done and there is discordance in genital appearance, one can suspect DSD. While the majority of DSD are diagnosed in the neonatal period, it may present in later life in boys with cyclical haematuria and breast enlargement. Girls may present with virilization of the external genitalia, primary amenorrhoea and inguinal hernia with gonad inside. Though the uterus can be palpated by rectal examination and mucus can be milked out, it may be traumatic. The uterus can be picked up by ultra sonogram.

Karyotyping, imaging especially ultrasonogram, genetic studies and hormonal profile, such as measurement of 17-hydroxyprogesterone, testosterone, gonadotropins, antimüllerian hormone, serum electrolytes, and urine analysis can be done in the preliminary stage.

The results of these investigations are generally available within 48 hours and will be sufficient to make a working diagnosis. ACTH and HCG stimulation tests will help to arrive at a differential diagnosis. Decision making algorithms are available to guide further investigation.

Once a basic diagnosis has arrived, further advanced investigative modalities can be planned such as genitogram, MRI studies, cystourethroscopy, gonadal and skin biopsy and in some cases laparoscopy both as a diagnostic and therapeutic modality. Genetic analysis though expensive is confirmatory especially in complete androgen insensitivity and 5 alpha reductase deficiencies etc. The diagnostic aspect of individual type of DSD is dealt with in the respective chapters.

References

1. Conn J, Gillam L, Conway G. Revealing the diagnosis of androgen insensitivity syndrome in adulthood. BMJ 2005;331:628–30.

2. Lee PA1, Houk CP, Ahmed SF, Hughes IA Consensus statement on management of intersex disorders. International Consensus Conference on Intersex. Pediatrics. 2006 Aug;118(2):e488-500.

Chapter 2

Gonadal Development and Defects in Testosterone Biosynthesis

Testicular Development

The germ cells develop in the extra-embryonic tissues surrounding the yolk sac and by 3rd to 5th week of development they migrate around the yolk sac into the urogenital ridge through the caudal umbilical stalk. The bipotential primitive gonad will become ovary by default. In the XY foetus the Y chromosome stimulates the testicular determining SRY gene and the primitive gonad will be converted into testis. It has Sertoli cells that produce Mullerian inhibiting substance (MIS) and the Leydig cells which produce Testosterone. Steroidogenic Factor 1 (SF1) is expressed in the bipotential gonad prior to the onset function of SRY. SF1 can upregulate SRY expression. Mutations in SF1 cause 46XY gonadal dysgenesis and also gonadal dysgenesis in 46XX individuals and result in primary ovarian insufficiency. Since the discovery of SRY gene in 1990, a number of other genes have been shown to be involved in testicular and ovarian development. Mutations in any one of these genes result in disruption of gonadal development.

The Mullerian inhibiting substance or Antimüllerian hormone (MIS / AMH) produced by the Sertoli cells prevents the development of Mullerian structures namely uterus and fallopian tube in the male child. The absence of MIS results in presence of uterus and the effect is unilateral and produces Persistent Mullerian Duct Syndrome (PMDS).

SOX-9 also plays a pivotal role in male sexual development by working with SF1. SOX-9 can produce AMH in Sertoli cells to inhibit the creation of a female reproductive system. SF1 (transcription factor) appears to be active in masculinising both the Leydig cells and Sertoli cells. In Sertoli cells with the SOX9 protein, it elevates the level of AMH transcription. In Leydig cells, it activates the gene encoding the enzyme that makes testosterone hormone. Next, Sox9 activates FGF9. Activation of FGF9 by SOX-9 starts vital processes in male development, such as the creation of testis cords and the multiplication of Sertoli cells. The association of SOX-9 and Dax1 actually creates Sertoli cells, another vital process in male development. The absence of FGF 9 even in individual with X and Y chromosome develop into a female. Suppression of WNT4 is required for male sex development. FGF signalling suppresses WNT4, acting in a feed forward loop triggered by SOX9. (Fig 1)

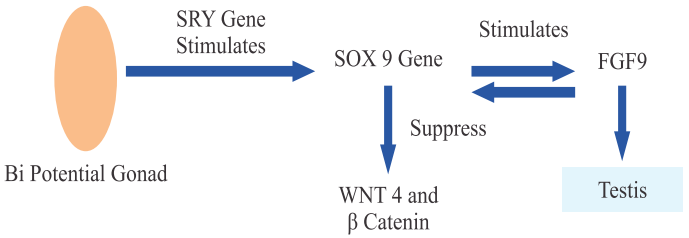


Fig. 1 - The Pathway of Testicular Development from Primitive Gonad

WNT4 is required for female sex development. Along with beta catenin, which increases the expression of target genes WNT4 is responsible for ovarian development. WNT4 also suppresses 5-α reductase activity, which converts testosterone into dihydrotestosterone. External male genitalia is therefore not formed. Moreover, it contributes to the formation of the Müllerian duct, a precursor to female reproductive organs. (Fig 2)

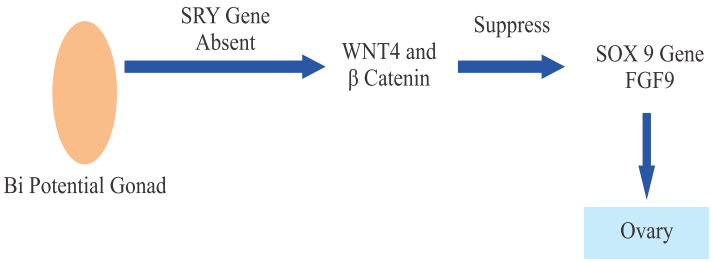


Fig.2 - The Role WNT4 in the Development of Ovary in the absence of SRY Gene

The Leydig cells which produce testosterone help in differentiation of Wolffian structures namely vas, epididymis and seminiferous tubules. These produce 5 alpha reductase which converts testosterone into Dihydrotestosterone (DHT). DHT is necessary for masculinization of the external genitalia, causing growth of the genital tubercle into a penis, and growth of the perineal body to cover the urogenital sinus, canalisation of the urethral plate to form the urethra, and fusion of the outer genital folds to form the scrotum. Absence of DHT results in small-sized penis, failure of labio scrotal folds to fuse and rudimentary vagina. Testosterone can be aromatised into estrogen in peripheral adipose tissue and other structures such as ovary, liver, brain, breast and metabolised in the liver and excreted by the kidney. Nearly 95 % of the testosterone is secreted by the testis and mostly bound to sex hormone binding globulin and nearly 3% is free testosterone. The other two androgens are androstenedione which also gets aromatised to estrogen and dehydroepiandrosterone a weak androgen which if increased as in 3b hydroxysteroid dehydrogenase deficiency in female children may produce virilization of external genitalia.

Apart from DHT which is necessary for external genitalia development in male children androgen receptor gene located on the X chromosome (locus: Xq11-Xq12) is necessary for normal external masculinisation. Mutation of the gene results in androgen

insensitivity syndrome. Androgen regulated genes are critical for the development and maintenance of the male sexual phenotype. (Fig. 3)

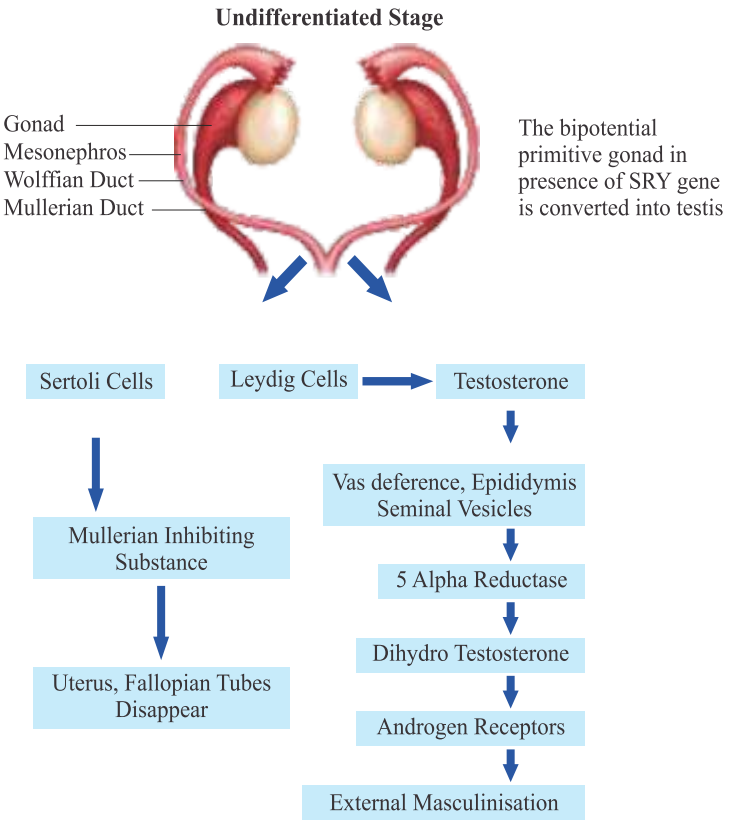


Fig. 3 - Flow Chart of Testicular Development

Testosterone Synthesis and Abnormalities.

Testosterone is synthesized from cholesterol through the action of various enzymes. Cholesterol also synthesizes Aldosterone and Cortisol. (Fig. 4)

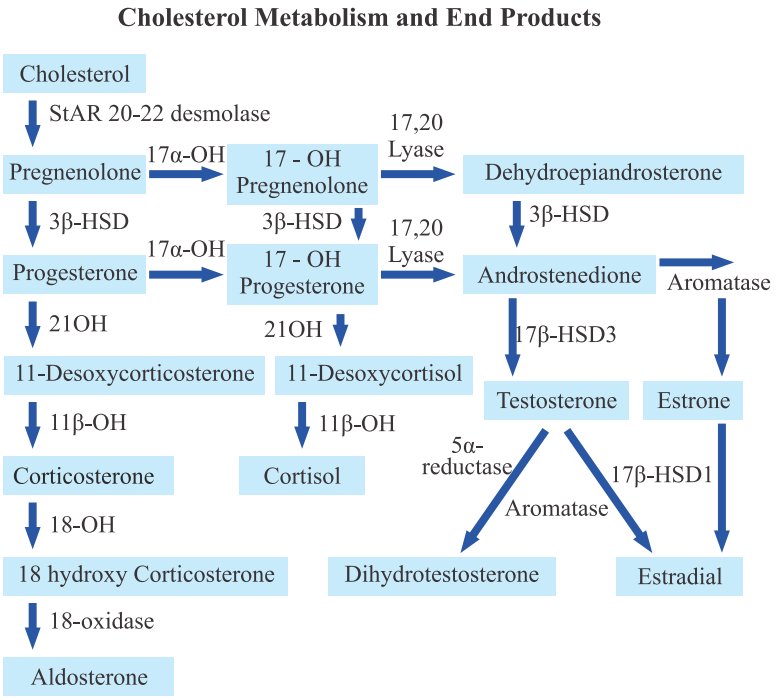


Fig. 4 - Flow Chart of Cholesterol Metabolism

Defect in Cholesterol Synthesis.

Defect in cholesterol synthesis leads on to Smith-Lemli-Opitz Syndrome (SLOS). It is an autosomal recessive, multiple malformation syndrome caused by a mutation in the enzyme 7-Dehydrocholesterol reductase encoded by the DHCR7 gene. The external genitalia in the male children are under virilized with micropenis bifid scrotum and hypospadiac urethra. The cholesterol level is low with an increase in 7- dehydrocholesterol. It causes a broad spectrum of effects, ranging from intellectual disability & behavioural problems to lethal malformation.

Since the report of Curry et al (1987), it has been recognized that many 46 XY individuals with severe manifestations of SLOS have extreme under masculinization of the external genitalia, resulting in female external genitalia (termed "sex reversal"[1]). Lin et al (1997) reported that 20%-25% of individuals with SLOS described in the literature have 46 XY karyotype with a female phenotype.[2]

Prenatally, SLOS is diagnosed upon finding an elevated 7DHC- total sterol ratio in fetal tissues, or increased levels of 7DHC in amniotic fluid. The 7DHC: total sterol ratio can be measured at 11–12 weeks of gestation by chorionic villus sampling and elevated 7DHC in amniotic fluid can be measured by 13 weeks.

Steroidogenic Acute Regulatory Protein (StAR) Mutations.

Congenital lipoid adrenal hyperplasia (lipoid CAH), the most severe form of CAH, is caused by mutations in the steroidogenic acute regulatory protein (StAR) encoded by a gene on chromosome 8p11.2 in the human.[3]

It is an autosomal recessive gene. Deficient fetal testicular steroidogenesis in patients with a 46 XY karyotype results in phenotypically normal female genitalia. The adrenal cortex becomes engorged with cholesterol and cholesterol esters; deficient adrenal steroidogenesis leads to salt wasting, hyponatremia, hypovolemia, hyperkalemia, acidosis, and death in infancy. The genitalia of XY foetuses with lipoid CAH are severely under virilized due to the impairment of steroid hormone synthesis. The fetal testes make AMH, which prevents a uterus and inner vagina from forming, but since the Leydig cells fail to make testosterone during development even in response to hCG, the testes usually remain in the abdomen. The formation of the penis, also dependent on testosterone, is compromised. Hence, the external genitalia in most of the infants resemble that of normal females (though the vagina is a short, blind

pouch), or is slightly ambiguous (more female than male). Nearly all reported XY cases have been assumed to be girls and raised as such with no reports of later gender identity problems.

Management does not differ from other forms of CAH and need both glucocorticoids and mineralocorticoids along with stress dosing.

17-Beta Hydroxysteroid Dehydrogenase Type 3 Deficiency

17-beta hydroxysteroid dehydrogenase type 3 (17βHSD-3) enzyme catalyse the conversion of androstenedione to testosterone (T) in the testes of the developing fetus, thus playing a crucial role in the differentiation of the gonads and in establishing the male sex phenotype. Any mutation in the encoding gene (17βHSD-3) can lead to varying degrees of under virilization of the affected male, ranging from completely under virilized external female genitalia to predominantly male with micropenis and hypospadias. Even those reared as females may virilize at puberty and may be difficult to differentiate it from partial androgen insensitivity and 5 alpha reductase deficiencies. HCG stimulation test may help to differentiate it from other types.[4] Those who are reared as girls gonadectomy is carried out to prevent virilization at puberty. Measurement of the testosterone-to-androstenedione ratio is useful to screen for 17βHSD3 deficiency. (<0.8), and genetic analysis can confirm the diagnosis. [5] Any young girl with an inguinal hernia, mild clitoromegaly, single urethral opening or urogenital sinus should raise suspicion. If not diagnosed early, patients present with severe virilization and primary amenorrhea in adolescence and may undergo a change from a female to male gender role.[6]

Isolated 17,20-Lyase Deficiency (ILD)

Isolated 17,20-lyase deficiency (ILD) is a rare autosomal recessive disorder with impaired production of the androgen and estrogen sex steroids. The condition manifests itself as partially or fully

underdeveloped males and in both sexes as reduced or absent puberty / lack of development of secondary sexual characteristics, resulting in a somewhat childlike appearance in adulthood (if left untreated).

Unlike the case of combined 17α-hydroxylase/17,20-lyase deficiency, isolated 17,20-lyase deficiency does not affect glucocorticoid production (or mineralocorticoid levels), and for that reason, does not result in adrenal hyperplasia or hypertension. [7]

The symptoms of isolated 17,20-lyase deficiency in males are ambiguity of external genitalia with micropenis, perineal hypospadias, and undescended testes and have a female gender identity & when reared as males gynecomastia develops.

Observed physiological abnormalities of the condition include markedly elevated serum levels of progestogens such as progesterone and 17α-hydroxyprogesterone (due to upregulation of precursor availability for androgen and estrogen synthesis), very low or fully absent peripheral concentrations of androgens such as dehydroepiandrosterone (DHEA), androstenedione, and testosterone and estrogens such as estradiol (due to the lack of 17,20-lyase activity, which is essential for their production), and high serum concentrations of the gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (due to a lack of negative feedback on account of the lack of sex hormones). [8]

Management

Those who are reared as females need removal of the testis and estrogen administration. Among those who are reared as males masculinisation of external genitalia, relocation of the testis to the scrotum and testosterone administration are necessary. [9]

17-Alpha Hydroxylase - 17 - 20 Lyase Deficiency

Combined 17alpha-hydroxylase/17,20-lyase deficiency (17OHD), caused by mutations in the CYP17A1 gene, is a rare autosomal recessive form of congenital adrenal hyperplasia and characterized by hyporeninaemic hypokalemic hypertension, primary amenorrhea and absence of secondary sexual characteristics. The above enzyme is necessary for the conversion of pregnenolone to 17 alpha hydroxy pregnenolone and finally converted into dehydroepi androstenedione. (Fig 5)

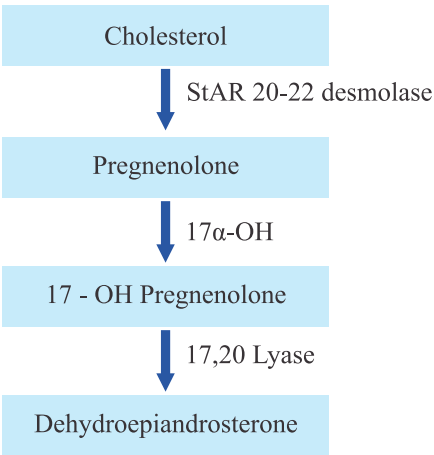


Fig. 5 - The Role of 17 Alpha - Hydroxylase / 17,20 - Lyase in Cholesterol Metabolism

The inheritance is an autosomal recessive gene where Mullerian duct structures are not present and Wolffian duct structures are either normal or hypoplastic. The gonads are either intra-abdominal or inguinal. External genitalia are feminine.

Most of them have hypergonadotrophic hypogonadism; uncontrolled hypertension; primary adrenal insufficiency; and high progesterone,

low to normal potassium, and low dehydroepiandrosterone, androstenedione, and testosterone levels. Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) at baseline and after an adrenocorticotropic hormone test show low cortisol and cortisone and high deoxycorticosterone (DOC) and corticosterone levels; both DOC/21-deoxycortisol and corticosterone/cortisol ratios are very high. [10]

Treatment primarily is control of hypertension which is achieved by the administration of glucocorticoids in the form of Hydrocortisone. They need removal of testis and estrogen supplementation if reared as girls. The majority are reared as girls and may present later in life as primary amenorrhoea.

3 Beta Hydroxysteroid Dehydrogenase.

3β-HSD II is necessary for the conversion of pregnenolone to progesterone, 17alpha hydroxypregnenolone to 17alpha-hydroxyprogesterone, dehydroepiandrosterone (DHEA) to androstenedione in the adrenals. 3β-HSD also mediates an alternate route of testosterone synthesis from androstenediol in the testes.

3β-HSD II deficiency results in large elevations of pregnenolone, 17alpha-hydroxy pregnenolone, and DHEA.

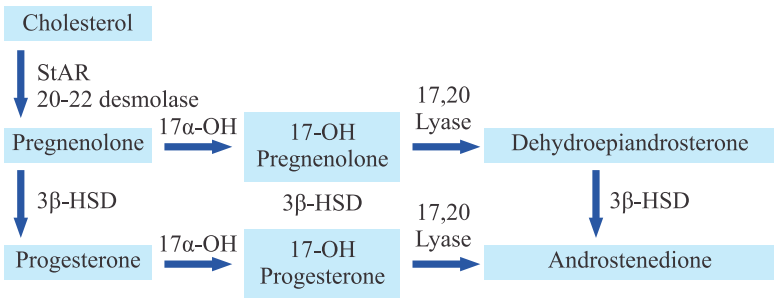


Fig. 6 - The Role of 3 Beta Hydroxysteroid Dehydrogenase in Cholesterol Metabolism

In the female the elevated DHEA produces mild virilization of the external genitalia. In the male the deficiency of 3β-HSD II produces under virilization of the external genitalia. If they are reared as girls, they virilize at the time of puberty due to testicular stimulation by high levels of LH. (Fig.6)

Management

If the boy is only mildly under virilized, the hypospadias can be surgically repaired, testes brought into the scrotum, and testosterone supplemented at puberty. In those who are grossly under virilized and reared as girls, need gonadectomy and vaginal lengthening, oestrogen supplements for breast development. A recently advocated third choice would be to assign either sex and defer surgery to adolescence. Each approach carries its own disadvantages and risks Fertility may be impaired by the difficulty of providing appropriate sex hormone levels in the gonads even though the basic anatomy is present.

References

1. Curry CJ, Carey JC, Holland JS, Chopra D, Fineman R, Golabi M, Sherman S, Pagon RA, Allanson J, Shulman S, et al. Smith-Lemli-Opitz syndrome-type II: multiple congenital anomalies with male pseudohermaphroditism and frequent early lethality. Am J Med Genet. 1987;26:45–57 (PubMed)

2. Lin AE, Ardinger HH, Ardinger RH Jr, Cunniff C, Kelley RI. Cardiovascular malformations in Smith-Lemli-Opitz syndrome. Am J Med Genet. 1997;68:270–8. [PubMed]

3. Bhangoo A, Anhalt H, Ten S, King SR (March 2006). "Phenotypic variations in lipoid congenital adrenal hyperplasia". Pediatr Endocrinol Rev. 3 (3): 258–71

4. Galli-Tsinopoulou A1, Serbis A1, Kotanidou EP1, Litou E1, Dokousli V1, Mouzaki K1, Fanis P2, Neocleous V2, Skordis N .46, XY Disorder of Sex Development due to 17-Beta Hydroxysteroid Dehydrogenase Type 3

Deficiency in an Infant of Greek Origin. J Clin Res Pediatr Endocrinol. 2018 Mar 1;10(1):74-78.

5. Grimbly C1, Caluseriu O2, Metcalfe P3, Jetha MM1, Rosolowsky ET1 .46XY disorder of sex development due to 17-beta hydroxysteroid dehydrogenase type 3 deficiency: a plea for timely genetic testing. Int J Pediatr Endocrinol. 2016;2016:12

6. George MM1, New MI, Ten S, Sultan C, Bhangoo A The clinical and molecular heterogeneity of 17βHSD-3 enzyme deficiency Horm Res Paediatr. 2010;74(4):229-40.

7. Stuart Handwerger (26 February 1999). Molecular and Cellular Pediatric Endocrinology. Humana Press

8. Kate-Booij MJ, Cobbaert C, Koper JW, de Jong FH (February 2004). "Deficiency of 17,20-lyase causing giant ovarian cysts in a girl and a female phenotype in her 46XY sister: case report". Human Reproduction (Oxford, England). 19 (2): 456–9.

9. Marschall Stevens Runge; Cam Patterson (20 June 2006). Principles of Molecular Medicine. Humana Press. p. 483.

10. Breder ISS, Garmes HM3, Mazzola TN2, Maciel-Guerra AT2, de Mello MPJ Pediatr Three new Brazilian cases of 17α-hydroxylase deficiency: clinical, molecular, hormonal, and treatment features, .Endocrinol Metab. 2018 Aug 28;31(8):937-942

11. Simard J, Moisan AM, Morel Y (August 2002). "Congenital adrenal hyperplasia due to 3beta-hydroxysteroid dehydrogenase/ isomerase deficiency". Semin. Reprod. Med. 20 (3): 255–76

Chapter 3

46 XX DSD
Congenital Adrenal Hyperplasia

The cause of ambiguity of external genitalia in female children is most often due to excessive androgens which causes virilization and rarely due to other causes mentioned below.

46XX DSD

The virilization of external genitalia in a girl child is due to one of the following conditions.

1. Congenital Adrenal Hyperplasia is the commonest cause of 46XX DSD
2. Maternal intake of hormones in the first trimester of pregnancy.
3. Maternal tumors like Arrhenoblastoma
4. Tumors of the clitoris.
5. Idiopathic

Congenital Adrenal Hyperplasia (CAH)

The commonest cause of virilization of the external genitalia in the girl child is due to CAH. It is an autosomal recessive disorder & results from inherited defects in one of the five enzymatic steps required for the biosynthesis of cortisol.

These disorders are so named because the adrenal glands are hyperplastic at birth due to unrestrained ACTH stimulation of the adrenal cortex in fetal life due to defective synthesis of steroids.

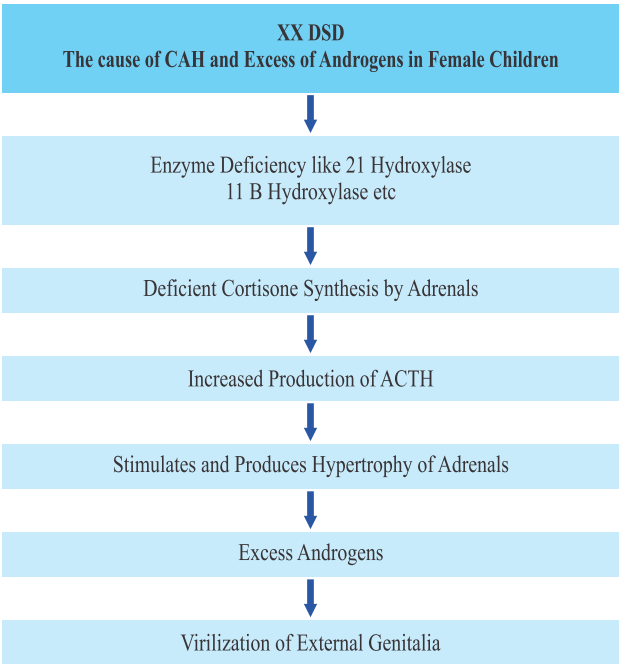


Fig. 1 - The Cause of Virilization in Congenital Adrenal Hyperplasia

Several enzymes are involved in the conversion of cholesterol to mineralocorticoids, glucocorticoids and sex hormones in the adrenal gland. Enzyme deficiency in the conversion of progesterone to glucocorticoids and mineralocorticoids results in inadequate levels of circulating steroids, with an increase in ACTH secretion which stimulates the adrenal cortex, causing hypertrophy.

The commonest enzyme deficiency is 21 Hydroxylase. (21-OHD) 21-OHD is caused by mutations in the CYP21 gene encoding the steroid 21-hydroxylase enzyme.[1] The activated adrenal can only produce androgen precursors due to enzyme deficiency; the precursors have masculinising effect on the female genitalia, giving rise to adrenogenital syndrome. The excess androgen affects the

developing genital tubercle and urogenital folds, which develop after 11 weeks; the virilization varying from simple clitoromegaly to gross ambiguity, with a male like phallus, fusion of labial folds and a long urogenital sinus.

The other enzyme deficiencies causing CAH are 17alpha-hydroxylase, 3Beta-hydroxysteroid dehydrogenase, and 11Beta hydroxylase and cholesterol desmolase. In 21 alpha hydroxylase and 11Beta hydroxylase deficiency only adrenal steroidogenesis is affected, whereas a defect in 3Beta hydroxysteroid dehydrogenase and 17alpha hydroxylase also involves gonadal steroid biosynthesis. (Fig 2)

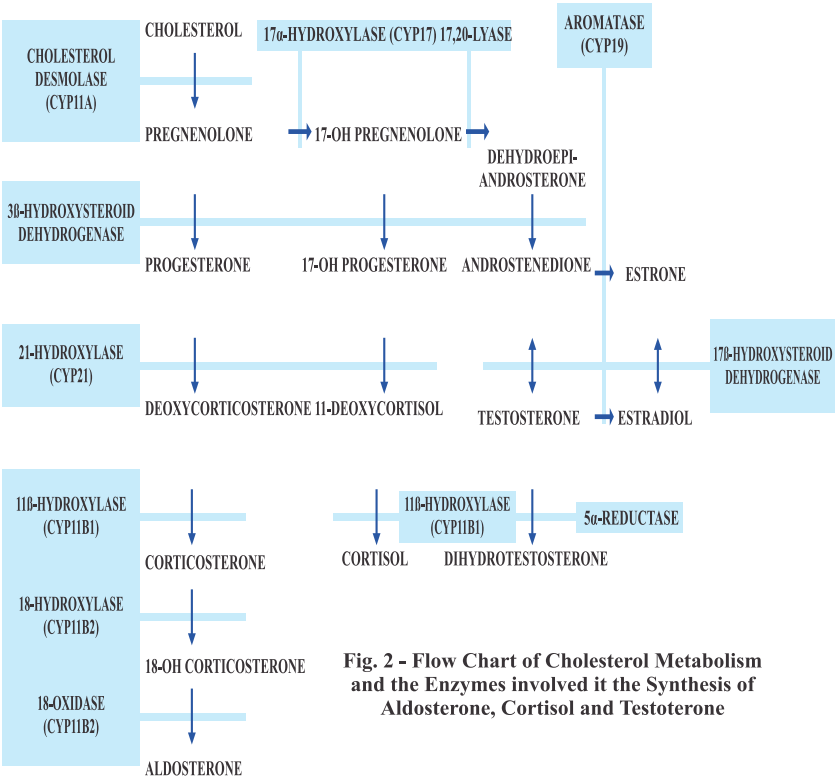


Fig. 2 - Flow Chart of Cholesterol Metabolism and the Enzymes involved it the Synthesis of Aldosterone, Cortisol and Testoterone

21 Hydroxylase Deficiency

21 hydroxylase deficiency, now known as CYP21, is an autosomal recessive condition and is the commonest enzyme deficiency found in almost 95% cases of CAH. Deficiency of CYP21 results in the deficiency of cortisol and excess of its precursor 17 hydroxy progesterone. This leads to elevated testosterone production causing a virilizing effect on the female genitals. About 75% of these patients also have salt loss due to inadequate levels of aldosterone, resulting in hyponatremia and hyperkalemia, which can be life threatening.

Clinical Presentation

Two varieties of CAH have been described: the classic and the nonclassic one also called as delayed onset CAH. The classic variety may be a salt loser or a nonsalt loser. Boys also suffer from this enzyme deficiency but do not have any ambiguity of external genitalia. The salt loser babies typically present in the neonatal period, between 1 to 4 weeks with vomiting and shock. Female baby shows varying degree of virilization along with hyperpigmentation of the genitalia and areola. Children with mild enzyme deficiency, (the nonclassic variety) present around puberty with hirsutism and or a sudden increase in the size of the clitoris.

Any child who has hypospadiac urethra bilateral empty scrotum with severe hyponatremia and vomiting should be investigated for CAH with ultrasound abdomen, karyotyping and 17OHP estimation. (Fig3)

Fig. 3 - Sunken eyes with severe dehydration with hypospadiac urethra in a girl child with CAH reared as a boy

The degree of masculinization of the external genitalia is variable and classified into five stages by Prader. Stage 1 applies to the presence of clitoromegaly, without labial fusion. Stage 2 describes clitoromegaly and posterior labial fusion. Stage 3 involves a greater degree of clitoromegaly with almost complete labial fusion and the presence of a urogenital sinus. In Stage 4 the clitoris has a phallic appearance, the urethral orifice is at the base of the clitoris and there is chordee with otherwise completely fused labial fold. Stage 5 describes a male phenotype due to the penile transformation of the clitoris and urethra and complete fusion of the labial folds. (Fig 4)

Fig. 4 - Stages of clitoromegaly from minimal enlargement of the clitoris to penile transformation of the clitoris

Such infants would be mistaken for under-developed males, but close examination reveals a lack of palpable gonads. The internal genitalia is normal for a female and pelvic sonography reveals a uterus, fallopian tubes and ovaries

Recently, Rink et al. developed the 'PVE' classification for genital ambiguity, where P represents stretched phallic length and width, V is the distance from the bladder neck to the vagina and the distance from the vagina to the perineal meatus, and E is the Prader number. This classification system was found to aid in the surgical planning and the analysis of surgical outcomes. [2]

Apart from fundamental abnormalities in adrenocortical steroid production, adrenomedulary function is also compromised due to developmental defects in the formation of the adrenal medulla, leading to decreased production of catecholamines, mainly epinephrine and may result in unstable cardiovascular status leading

to shock and the characteristic 'adrenal crisis'. It has even been proposed that measuring plasma free metanephrine and molecular genotype predict phenotype with similar accuracy. [3,4]

Nonclassic CAH Phenotypes

CAH may present as late onset clitoromegaly in pubertal age or later period. The clinical signs (premature pubarche, and advanced bone age, menstrual disturbances, infertility, slowly, progressive hirsutism, acne) occur at later childhood, adolescence or after puberty. (Fig 5)

Fig. 5 Late onset of CAH. Hirsutism and clitoromegaly are present

The pathophysiology of the less frequent and milder reproductive problems associated with non classic CAH is presumably similar to that suggested for classic CAH. Data regarding reproductive function in non classic CAH come mainly from studies of populations referred for symptoms and signs of hyperandrogenism and or infertility; ascertainment bias obviously affects such studies. In one report 39% of women presented with hirsutism, 39% with oligomenorrhea or other signs of polycystic ovaries, and 22% with no obvious signs of androgen excess. [5]

The affected females have adrenal steroid precursors of 21-hydroxylase deficiency but they are only mildly elevated in nonclassic CAH. Affected individuals have serum 17-OHP levels of greater than 1,000 or 1,500 ng/dl 60 min after an intravenous bolus of cosyntropin (ACTH 1–24).

Carrier State Detection

Asymptomatic parents, siblings who are carriers of CAH were detected by HLA in the past and by DNA now. Synthetic ACTH test may show marginally elevated levels of 17 OHP compared to normal individuals. A rise in 21-deoxycortisol levels done at the same time is very useful to detect the heterozygous state in the family members of CAH patients and in the general population and will be more specific.

Diagnosis and Investigations

Clinical Examination

When the physical examination shows enlarged clitoris, vaginal and urethral orifices separately and the lack of gonads in labia and inguinal canals, one must suspect CAH. The degree of external genitalia masculinisation may vary as described earlier.

Laboratory Investigations

- Ultrasound examination for internal genitalia and gonads if any
- Fluorescent in situ hybridization for SRY Gene / Karyotype
- Estimation of 17 OHP, Testosterone, FSH, LH.
- Serum electrolytes
- ACTH stimulation and HCG stimulation and estimation of the above.
- Genetic studies
- Genitogram and MRI in selected cases
- Gonadal biopsy either by laparoscopy or open surgery if needed

Standard diagnostic tests should include at least a measurement of basal serum 17-OHP, but preferably a complete profile of adrenocortical hormones before and one hour after cosyntropin stimulation. These assays should be done after the first 24 hours of life. They will identify potential defects in adrenal steroidogenesis.

Salt wasting 21-hydroxylase deficiency is the most commonly encountered cause of CAH. After testing is completed, the child's vital signs should be monitored for any indication of an adrenal crisis. It is rare for salt wasting crisis to occur before 7 days of life, but many clinicians will obtain electrolyte measurements to assess hyponatremia and hyperkalemia in CAH newborns during the first week. Plasma renin activity and aldosterone are elevated in many normal infants and do not usually add much useful information within the first days of life. In neonates presenting in shock, serum electrolytes along with 17hydroxyprogesterone (17OHP) levels will reveal hyponatremia, hyperkalemia and excessive levels of 17OHP.

There is a rise of 17 OHP in premature children in the immediate postnatal period and hence the 17 OHP estimation is done after 48 to 72 hours. It can be done by the heel prick method and recently, from the saliva of the neonate. Ideally, to fully differentiate the various enzymatic defects, the clinician should measure 17-OHP, cortisol, desoxycorticosterone acetate (DOC) 11-deoxycortisol, 17-OH-pregnenolone, dehydroepiandrosterone (DHEA), and androstenedione at 0 min and 60 min after cosyntropin test. It is suggested that genotyping is done only when results of the adrenocortical profile after a cosyntropin stimulation test are equivocal or for purposes of genetic counselling.[6] The Karyotype of these children who do not have palpable gonads show 46XX.

The diagnosis of CAH is based on clinical examination of external genitalia whether the gonads are palpable or not and the ultrasound examination of the abdomen to see the uterus and biochemical examination of the blood for various hormones and the precursors. The following flow chart is working diagnosis in a neonate with ambiguity of external genitalia. (Fig 6)

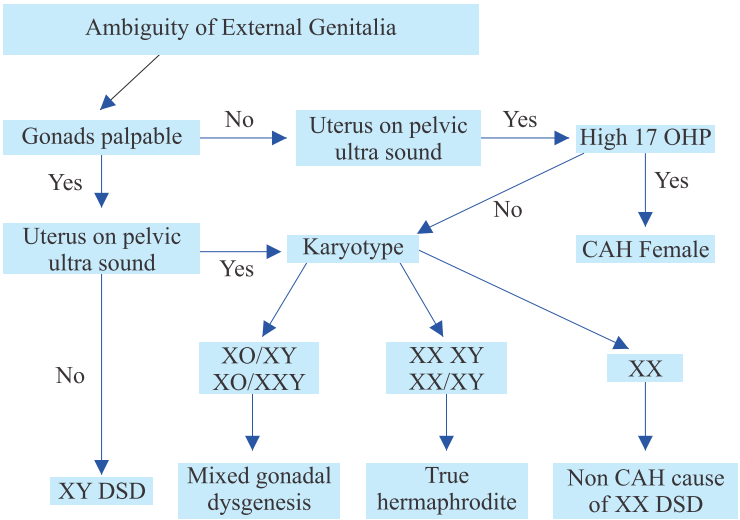


Fig. 6 - Flow Chart of working Diagnosis in a Child with DSD

Synthetic ACTH Stimulation (Synacthen Test)

It also helps to differentiate other types of CAH particularly 3 Beta hydroxysteroid dehydrogenase (3BetaHSD) deficiency.

Dehydroepiandrosterone is increased several folds in Synacthen test in children born with 3Beta HSD deficiency who also have deficiency of cortisol and aldosterone.
(Fig 7)

Fig. 7 - Hyperpigmented and virilized twin girls with 3Beta HSD deficiency showed markedly increased dihydro epi androstenedione (DHEAS) on Synacthen Test

DHEAS mcg/dl	First Twin 4536	Second Twin 5406	Normal Range 10-248 (Birth to 5 days)

There are a group of children who have minimal clitoral hypertrophy whose 17 OHP levels are normal at birth and labeled as idiopathic. However, they may show virilization as they grow older. These are CAH-children with simple virilization and can be identified by synthetic ACTH stimulation which will show higher levels of 17 OHP progesterone. (Fig 8)

Fig 8. Considered as Idiopathic Clitoromegaly, showed raised 17 OHP levels on Synacthen Test

11b-Hydroxylase Deficiency

The second most common enzyme deficiency is 11b-hydroxylase. The characteristic feature is the accumulation of desoxycorticosterone that causes salt retention and hypertension. Virilization of the external genitalia varies from minimal phallic enlargement to almost complete masculinisation. Because of the adrenocorticotropic hormone drive there is hyperpigmentation of the external genitalia and nipples. A female gender is preferred in those with a karyotype of 46XX. Fertility is normally expected. Diagnosis of 11beta-hydroxylase deficiency in neonates is established by increased plasma levels of 11-deoxycortisol and adrenal androgens (DHEA, androstenedione, and testosterone). Plasma renin activity is often suppressed because of increased mineralocorticoid activity; this test may be useful in older children but is less reliable in neonates. If the diagnosis is uncertain, levels of 11-deoxycortisol and adrenal androgens are measured before and 60 min after ACTH stimulation. In affected adolescents, basal plasma levels may be normal, so ACTH stimulation is recommended.

Treatment is cortisol replacement, typically with hydrocortisone 3.5 to 5 mg/m2 tid, with total daily dose typically ⩽ 20 mg/m2, which prevents further virilization and ameliorates hypertension by reducing levels of 11-deoxycortisol, deoxycorticosterone, and adrenal androgens that are stimulated by ACTH. Unlike 21 hydroxylase deficiency. mineralocorticoid replacement is not required because sodium and potassium homeostasis is maintained from mineralocorticoid effects of deoxycorticosterone.

Response to treatment should be monitored, typically by measuring serum 11-deoxycortisol and adrenal androgens and by assessing growth velocity and skeletal maturation. BP should be monitored closely in patients who presented with hypertension. Antihypertensives, such as potassium-sparing diuretics or calcium channel blockers, may be required. [7]

3β Hydroxy Steroid Dehydrogenase Deficiency

3βHSD deficiency affects all three biosynthetic pathways (mineralocorticoids, glucocorticoids, sex steroids).[8] The clinical spectrum shows a wide variety of disease expression, ranging from a severe salt wasting form and a non salt wasting form, with or without ambiguous genitalia in affected male neonates, With severe deficiency, the most common presentation is that of a newborn infant with adrenal insufficiency due to both glucocorticoid and mineralocorticoid deficiency and ambiguous genitalia in 46XY patients. Infants with less severe (non–salt-wasting) forms may be relatively asymptomatic. 3β-HSD deficiency results in large elevations of pregnenolone, 17α-hydroxypregnenolone, and Dehydro epiandrosterone (DHEA). In an XX (genetically female) fetus, elevated amounts of DHEA can produce moderate virilization by conversion in the liver to testosterone. Virilization of genetic females is partial, often mild, and rarely raises assignment questions. The issues surrounding corrective surgery of the virilized female genitalia

are the same as for moderate 21-hydroxylase deficiency but surgery is rarely considered desirable. Under virilization of genetic males with 3β-HSD, CAH occurs because the synthesis of testosterone is impaired in both adrenals and testes. Although DHEA is elevated, it is a weak androgen and too little testosterone is produced in the liver to offset the deficiency of testicular testosterone. The degree of under virilization is more variable, from mild to severe.

Management consists of replacing mineralocorticoid for salt losers with fludrocortisone, suppressing DHEA and replacing glucocorticoid and providing extra glucocorticoid for stress. In male children the management issues are those of an under virilized male with normal sensitivity to testosterone.

17α - Hydroxylase Deficiency

Congenital adrenal hyperplasia due to 17α-hydroxylase deficiency is an uncommon form of congenital adrenal hyperplasia resulting from a defect in the gene CYP17A1, which encodes for the enzyme 17α-hydroxylase. It produces decreased synthesis of both cortisol and sex steroids, with a resulting increase in mineralocorticoid production. Thus, common symptoms include mild hypocortisolism, ambiguous genitalia in genetic males or failure of the ovaries to function at puberty in genetic females, and hypokalemic hypertension. Affected genetic (XX) females may be wholly asymptomatic except for infertility. [9]

CAH caused by P450 Oxido Reductase Deficiency (ORD) (apparent combined CYP17A1 and CYP21A2 deficiency).

Typical findings include raised 17OHP, albeit not to the extent observed in 21OHD. In contrast to 21OHD, sex steroids are low and there is no mineralocorticoid deficiency.

The combined partial inhibition of 17α hydroxylase and 21 hydroxylase results in glucocorticoid deficiency in most but not all

affected children. Baseline glucocorticoid secretion is often still sufficient; however, the cortisol response to stress or ACTH stimulation is usually impaired.[10]

Therefore, affected children always require an assessment of adrenal function and in case of a failed Synacthen test. Hydrocortisone replacement needs to be provided at least in severe stress, illness or during surgery. As there is no postnatal androgen excess, affected children usually require lower doses of glucocorticoids than children with 21OHD since hydrocortisone treatment in 21 hydroxylase deficient patients not only replaces glucocorticoids but also controls adrenal androgen excess. There is strong evidence that affected patients without hydrocortisone replacement are at a high risk for developing a life-threatening adrenal crisis.

Skeletal abnormalities observed in the context of ORD predominantly comprise craniofacial malformations, specifically midface hypoplasia with low set ears and a pear shaped nose. Craniosynostosis is observed in variable degrees and requires ventriculoperitoneal shunts in more severely affected cases. ORD phenotype comprises a striking apparent contradiction, with clinical evidence of prenatal androgen excess in females but potential prenatal androgen deficiency in males combined with postnatal androgen deficiency in both sexes. Arlt et al. have proposed that this apparent contradiction is explained by the existence of an alternative pathway towards androgen synthesis, present during human foetal life only. [11,12]

Treatment options are glucocorticoid replacement therapy for cortisol deficiency including stress-dose cover in intercurrent illness; surgery is needed for craniosynostosis, hypospadias, and cryptorchidism in males and clitoromegaly and vaginal hypoplasia in females. Dihydrotestosterone treatment has been successful in some males with micropenis; testosterone replacement in males in whom testosterone levels remain relatively low after onset of puberty;

females with absent pubertal development may require estrogen replacement therapy. [13]

Lipoid Adrenal Hyperplasia

Congenital lipoid adrenal hyperplasia (lipoid CAH), the most severe form of CAH, is caused by mutations in the steroidogenic acute regulatory protein (StAR). Lipoid CAH is common among the Japanese, Korean, and Palestinian Arab populations, have a severe defect in the conversion of cholesterol to pregnenolone, the first step in adrenal and gonadal steroidogenesis.[14]

Deficient fetal testicular steroidogenesis in patients with a 46XY karyotype results in phenotypically normal female genitalia. The adrenal cortex becomes engorged with cholesterol and cholesterol esters. Deficient adrenal steroidogenesis leads to salt wasting, hyponatremia, hypovolemia, hyperkalemia, acidosis, and death in infancy.[15] Patients can survive to adulthood with appropriate mineralocorticoid and glucocorticoid replacement therapy.[16]

Some affected infants have immediate signs of mineralocorticoid deficiency, but others remain asymptomatic for months; furthermore, the affected 46XX females may undergo feminization and have vaginal bleeding at puberty Thus, it is not known whether the congenital lipoid adrenal hyperplasia syndrome is a single disease, or how a single genetic defect could account for these clinical variations.

The diagnosis of lipoid CAH must be distinguished from other combined glucocorticoid and mineralocorticoid deficiencies The distinction between lipoid CAH and 21-hydroxylase deficiency is simple; patients with lipoid CAH have female external genitalia regardless of karyotype and have very low or unmeasurable levels of all steroid hormones whereas patients with 21-hydroxylase deficiency have high concentrations of 21-deoxysteroids, especially 17-hydroxyprogesterone, and affected 46XX individuals are

virilized. Clinical, imaging and hormonal findings alone may not distinguish between P450scc and StAR deficiency; gene sequencing is the only definitive diagnostic method.[17]

Treatment is the same as other forms of CAH with hormonal replacement therapy with physiological doses of glucocorticoids and mineralocorticoids. [14]

Imaging in CAH

Ultrasound imaging of the abdomen will show the uterus and fallopian tubes and genitogram will show whether the urogenital sinus (UGS) has low or high confluence and a well-conducted genitogram shows important anatomical details that can be used in operative planning and eliminates the need for an endoscopic examination in a separate session. (Fig 9)

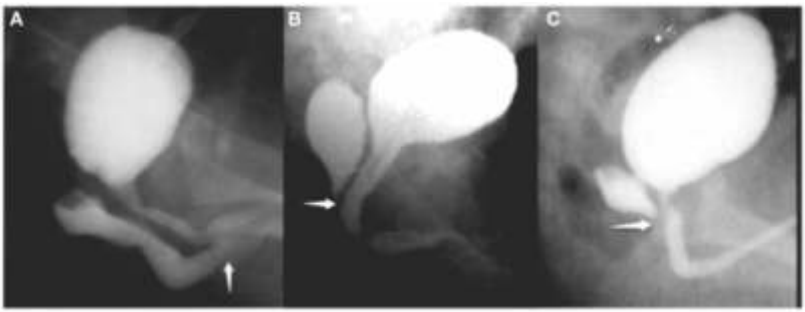


Fig. 9 - Genitography is very useful for the study of vaginal morphology, dimension and relation to the urethra. Depending on the vaginal confluence in the UGS classified as

(A) Low where the vagina opens low down in the urogenital sinus
(B) Intermediate, where the vagina opens in the middle of the urogenital sinus and
(C) High variant where the vagina opens beneath the internal meatus of the bladder
(With permission from Frontiers in pediatrics)

Genitoscopy at the time of surgery or earlier helps to further define the anatomy of the urogenital sinus. Because of radiation exposure and the need for sedation the use of CT and MRI should be reserved for patients with suspect or inconclusive findings on ultrasonography.

Laboratory Evaluation

Firstline testing in newborns includes karyotyping with X and Y specific probe detection (even when a prenatal karyotype is available). The results of karyotyping can take 2 - 3 days. Fluorescence in situ hybridisation can be used to identify X and Y chromosomes within a few hours. The laboratory evaluation should also include an assay for serum electrolytes, to exclude a salt-wasting form of CAH, a measurement of 17-hydroxyprogesterone (usually after 48 hrs to avoid interference with maternal progesterone), testosterone, gonadotropins, and Mullerian inhibiting substance (MIS). Urine analysis is also required to exclude proteinuria associated with Denys Drash syndrome.

A biopsy as well as laparoscopy is not required when the diagnosis is clearly established biochemically or by gene studies, as the histology can be confidently predicted. It is only required when an ovotestis or dysgenetic gonad is suspected to determine the definitive diagnosis. Newborn screening by 17OHP measurements from filter paper was first introduced by Pang et al.[19] There is a worldwide consensus that neonatal mass screening for 21hydroxylase deficiency is a mandatory tool for identifying affected children in the early neonatal period .[20] This prevents incorrect gender assignment in females and life threatening salt wasting crisis in both sexes. The decrease in mortality and morbidity subsequent to the introduction of neonatal screening has been demonstrated by several groups and also illustrated by an equalization of the female to male ratio in newly diagnosed cases following the implementation of screening. A female preponderance of 21OHD is still reported in the UK. The implementation of a neonatal mass screening program for CAH should be a future goal in paediatrics and in particular paediatric endocrinology. [21]

Treatment of congenital adrenal hyperplasia:

Glucocorticoids should be given in the appropriate doses to suppress ACTH and adrenal androgens without suppressing growth. Hydrocortisone is the preferred steroid in children with CAH and used in a dose of 10-15 mg/m2/ day. Recently diagnosed CAH in neonates need higher doses to suppress their hyperactive CRH- ACTH - adrenal axis.

Mineralocorticoid therapy returns plasma volume to normal in children with CAH. It removes the stimulation of ACTH by hypovolemia. Fludrocortisone is the mineralocorticoid used in the dose of 150-300 micg/day in newborns along with salt supplementation. The dosage in older children is 50-150 micg/day. A few older patients may be able to stop fludrocortisone due to free access to salty food, due to the mineralocorticoid function of their glucocorticoids and increased sensitivity to mineralocorticoids.

The need for stress dosing has to be explained in detail to families. The regular oral dose of hydrocortisone is doubled or tripled and given every six hours during periods of stress. If the child is unable to take hydrocortisone orally, it has to be delivered intravenously or intramuscularly. During adrenal crises, hypoglycaemia and hypotension can be managed with dextrose and normal saline infusion along with hydrocortisone bolus + infusion.

Periodic assessment of children with CAH is performed every 3 months with monitoring of growth, puberty, 17OHP and testosterone. Bone age assessment should be undertaken at least once a year.

GnRH agonist treatment may be necessary in poorly compliant children with true central precocious puberty. Bilateral laparoscopic adrenalectomy may be helpful in children with poorly controlled severe CAH requiring very high doses of glucocorticoids. Certain special situations may warrant treatment of CAH patients with anti-

androgens, aromatase inhibitors or growth hormone therapy. Antenatal treatment of pregnant heterozygous mother with a heterozygous partner is highly controversial as there is seven in eight chance of unnecessary exposure of a fetus to high dose steroids to prevent virilization.

Newer preparation of hydrocortisone, 'Chronocort' has been formulated to closely mimic the physiological pattern of normal circadian secretion of cortisone.

The treatment challenge lies in maintaining a fine balance so as to prevent hypercortisolism and hyper androgenisation. Undertreatment carries the risk of adrenal crisis and allows increased adrenal androgen production, with accelerated bone age and loss of growth potential; overtreatment may suppress growth, increase blood pressure, and cause iatrogenic Cushing's syndrome.

The goal of therapy is to use the smallest possible dose to suppress androgens and maintain growth. Despite the advances, the clinical management of patients with CAH is often complicated by abnormal growth and development, iatrogenic Cushing's syndrome, inadequately treated hyperandrogenism, and infertility. However, with proper compliance of intake of drugs and periodical assessment by paediatric endocrinologist the above complications can be prevented and they attain normal puberty and can be fertile and bear children.

Clinical Practical Guidelines

The Endocrine Society of Clinical practical guidelines suggest that in patients less than 18 months with CAH regular monitoring in the first 3 months of life and every 3 months thereafter. After 18 months, it is recommended evaluation every 4 months. It also recommends regular assessment of growth velocity, weight, blood pressure, as well as physical examinations in addition to obtaining biochemical

measurements to assess the adequacy of glucocorticoid and mineralocorticoid. In paediatric patients with congenital adrenal hyperplasia, it is advised to have annual bone age assessment until near-adult height is attained. In patients with congenital adrenal hyperplasia on treatment, should always wear or carry medical identification indicating that they have adrenal insufficiency. [22]

Medical Management and Clitoromegaly

Even what appears to be severely virilized CAH undergoes a significant reduction in the size of the clitoris with adequate treatment. The response is better in infancy than in older children. (Fig 10)

Fig. 10 - Markedly virilized girl infant with complete regression in size of clitoris after 8 months of steroid therapy (Dr. Ahila)

Children in whom the clitoral regression is not adequate and have a social problem in crèche or school clitoroplasty is advised. British association of pediatric surgeons have recommended the following guidelines on clitoroplasty.

- Clitoral Surgery should be avoided on mild and moderately virilized children.
- Clitoral surgery on severely virilized children must be carefully discussed with all involved with full understanding of effects on the future.
- The possibility of deferring surgery should be discussed with the parents.
- The possible requirement for further revision surgery must be recognized.

However, surgery may be offered to virilizing type of congenital hyperplasia in a school going girl child with obvious androgenisation with social embarrassment, informed consent must be obtained and clitoroplasty can be done (Fig 11).

Fig. 11 - Clitoroplasty for a school going child with clitoromegaly and social embarrassment

In those where urethra and vagina are not separate and have common urogenital sinus surgical treatment is focused on restoring normal genitalia anatomy. This is achieved by bringing the vagina to the normal position on the perineum, separating the distal vagina from the urethra, forming a normal introitus and preserving sexual function of the clitoris by accepting moderate degrees of hypertrophy as normal and strategically reducing clitoral size only in the most severely virilized patients. [23]

The main surgical principles are 1) Removing the corpora and preserving the glans with its innervation to create a clitoris with normal sensation 2) Creating a normal-appearing introitus by fashioning labia minora from phallic skin and foreskin and 3) Vaginoplasty to provide an adequate opening for the vagina onto the perineum.

Benefits of Early Surgery

It carries the benefit of rearing the child with a specific diagnosis and allows them to grow normally which has a tremendous psychological advantage with near normal appearing genitalia. The stigma attached

to DSD is not there and the parental anxiety is also minimised. The surgery done in childhood lessens the long-term memory of the procedure done in infancy and early childhood and allows the child to grow normally with her peers.

The combination of satisfactory cosmetic and functional outcomes and gender identity is required for sexual activity and sexual satisfaction in later life. It normalises voiding function and menstruation and prevents virilization at puberty. If the vagina has been reconstructed adequately it allows penetrative intercourse and fertility. They get adjusted psychologically when they reach puberty.

Risks of Early Surgery

It needs to be done by experienced person or team. Otherwise this adversely affect genital sensation sexual satisfaction, orgasm and may induce fear of penetrative sex especially when the child goes through repeated vaginal dilatation for vaginal stenosis. Primary surgery may lead to multiple complicated surgeries with impairment of fertility if not performed properly.

Types of Clitoroplasty and Vaginoplasty

Historically, clitoral reconstruction has progressed through stages of clitorectomy, followed by clitoral recession, and now, most commonly, clitoral reduction. Clitorectomy results in the obvious loss of sensation and may jeopardize the ability of an orgasmic response to tactile sensation. Clitoral recession maintains sensation; however, pain secondary to clitoral engorgement at the time of sexual arousal can occur. The most reasonable procedure for performing clitoroplasty is based on the concept of maintaining the clitoral glans and sensory input, which facilitates orgasm. The technique consists of the resection of the corpora at the crura with careful preservation of dorsal nerves and vessels and ventral mucosa that supply the glans. (Fig 12)

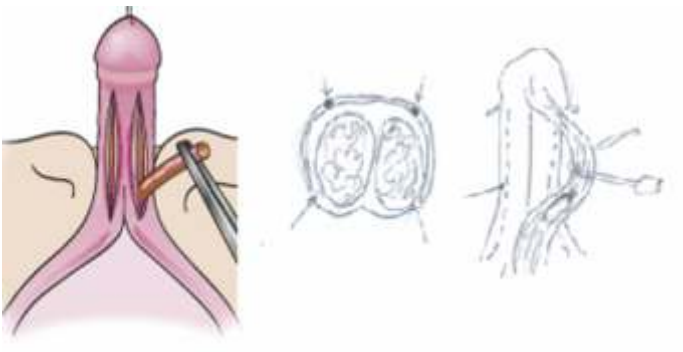


Fig.12 - Reduction clitoroplasty where the neurovascular bundle is preserved and corpora resected

In corpora preserving clitoroplasty by Pippi Salle, the corpora are detached from the glans, divided and fixed on either side of the labia.[24] Fig (13)

Fig. 13 - Corpora preserving clitoroplasty (Pippi Salle)
A. The corpora dissected from the neurovascular bundle
B. The corpora is divided into two halves
C. The prepuce of clitoris is divided in the midline to create labia minora
D. Final appearance after clitoroplasty (Dr. Sudipta Sen)

In corpora preserving clitoroplasty if the child is noncompliant with drugs, stump hypertrophy and painful erections develop. Hence resection of the corpora with glans preservation is the most acceptable

method of clitoral reconstruction. Gross hypertrophy of corpora in corpora sparing clitoroplasty (Pippi salle method) and stump hypertrophy and painful erection occurs in those who were noncompliant to steroid replacement. (Fig 14)

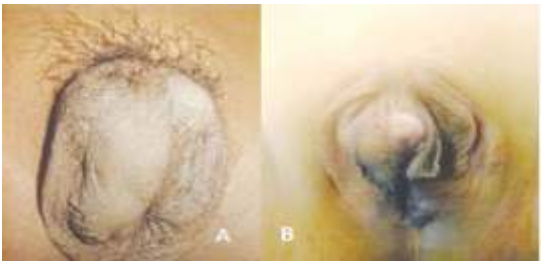


Fig. 14 - A. Gross hypertrophy of corpora in labia in corpora preserving clitoroplasty B. Stump hypertrophy in recession clitoroplasty

Based on his observation of innervation of clitoris Dr. Baskin suggested ventral resection of large glans clitoris where the nerve supply is least. Care is taken to preserve all nerves. Reduction of the glans clitoris should not violate the extensive innervation that predominates on the dorsal aspect of the glans. [25,26]

Clitoroplasty alone is adequate when the urethra and vagina are separate as in Prader stage 1 defect. The vagina may join the urethra high up close to the bladder or low down near perineum and called high and low confluence respectively with a common urogenital sinus.

Fortunately, lower confluence is more common than the higher ones. Higher the confluence, management is technically more complex and the reconstruction carries greater risks. The relationship of the vagina to the bladder neck is the most critical determining factor in the type of vaginoplasty to be performed. Cystoscopy can be done as part of the initial diagnostic evaluation in complex UGS abnormalities, but most often can be done at the time of reconstruction in those with CAH, avoiding additional anaesthesia. However, the relationship of the

vagina to the urethra can be identified by properly done genitography, as shown in Fig. 9.

In the low confluence of the urogenital sinus, a limited dissection of urogenitalsinus with posterior vaginal wall mobilisation is enough to bring the urethra and vagina to the exterior. (Fig 15)

Fig. 15 - Limited posterior vaginal wall mobilisation for low confluence

In the high confluence vagina, the options of management are total urogenital mobilisation (TUM) and partial urogenital mobilisation. In TUM the urogenital sinus is dissected circumferentially and mobilized. The dissection is carried through the pubourethral ligament anteriorly behind the pubic bone and posterior to the vagina allowing the entire UG complex (sinus, urethra, bladder, vagina) to move toward the perineum.[27] Good results especially on continence of bladder have been reported by the TUM method by several authors.[28,29] However, Sitites J observed that vaginoplasty in patients with congenital adrenal hyperplasia with TUM carries a risk of severe incontinence and may require further procedures to achieve urinary continence.[30]

The fear of incontinence has led to modifications where the mobilisation of the urogenital sinus stops at the urogenital membrane since the vast majority of patients do not need the more aggressive complete mobilization but can be managed by partial urogenital mobilisation (PUM) stopping the dissection at the pubourethral ligament.[31] (Fig 16)

Fig. 16 - Partial urethrovaginal mobilisation up to pubourethral membrane

Vaginoplasty done in infancy needs periodical dilation to prevent stenosis. The need for the vagina is for menstruation and sex which is not a priority in infancy. Hence in the high confluence where the vagina opens high up, the vaginoplasty is done at 10 years of age and beyond. Crouch NS found 77% needed revision in those who had early reconstruction.[32]

In older children where primary vaginal pull through is not done in infancy or failed pull through, the vaginal mobilisation is done through the abdomen and if the vaginal length is adequate primary pull through of the vagina is done after disconnection from the urogenital sinus. (Fig17a) However, if the vagina is short and high up, colo-vaginoplasty is done. (Fig .17b)

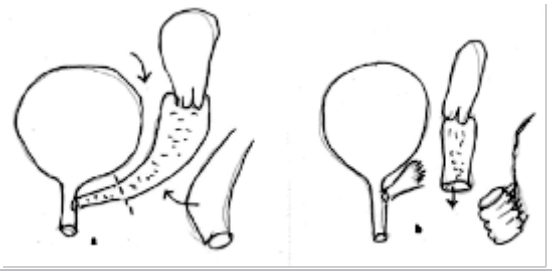


Fig. 17 a - b

Snyder did Y-V plasty technique to open the urogenital sinus, without having a separate vaginal orifice with good results even in cases of high vaginal insertion. When the vaginal introitus could not be reached, the urogenital sinus was surgically enlarged using the Y-V plasty technique. The absence of a separate vaginal opening did not impair sexual intercourse or cause urinary tract infections. The high vagina usually drained well via a urogenital sinus and rarely pooled sufficient urine to be a source of infection or interfere with normal bladder emptying.[33]

CAH and Pregnancy

Fertility is classically reported as low in CAH female patients especially among women with the salt-wasting variant. Several factors have been suggested to contribute to this impaired fertility: adrenal overproduction of androgens and progestins, 17-OHP & progesterone ovarian hyperandrogenism, Polycystic ovarian syndrome, ovarian adrenal rest tumours, neuroendocrine factors, genital surgery, and psychological factors such as delayed psychosexual development and reduced sexual activity. However, when CAH is not well controlled with medications, the overproduction of male sex hormones may interfere with ovulation and prevent pregnancy from occurring. Adjustment of the steroid dose can often lead to the restoration of ovulation. Proper follow up with endocrinologists with adjustments of steroids is necessary. CAH if properly treated, is compatible with normal fertility and pregnancy. (Fig 18)

Fig. 18 - CAH Mother Treated from Infancy has phenotypically Normal Child

Although low fertility rates have traditionally been reported among women with classic CAH, more recent data suggest that fertility rates are significantly improved, largely owing to earlier treatment of CAH, improved compliance with therapy, and surgical advances in genital reconstruction. Furthermore, ovulation induction and assisted reproductive techniques are now available to women who remain infertile despite effective adrenal androgen suppression. Although the pregnancy experience in women with classic CAH remains limited, it is apparent that once pregnant, these women have a high probability of a successful outcome.[34]

Children born to mothers of CAH with normal father will have a normal phenotype but will be carriers of the CAH gene. (Fig. 19)

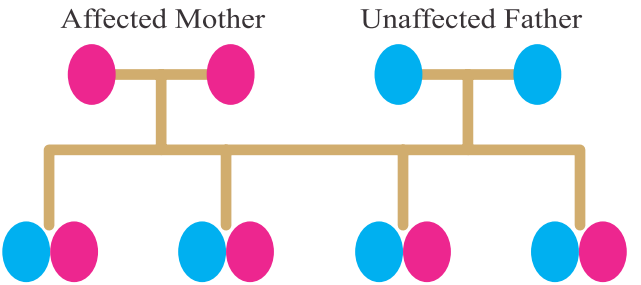


Fig. 19 - All children born to CAH mother will be a carrier of CAH recessive gene

Sports and Gender

Female athletes who have high androgen levels face the problem of getting disqualified from the sports events. The most recent IOC recommendations, as defined by a group of experts, are as follows: (1) A female recognised in law should be eligible to compete in female competitions provided that she has androgen levels below the male range (as shown by the serum concentration of testosterone) or, if within the male range, she has an androgen resistance such that she derives no competitive advantage from such levels. (2) An evaluation

with respect to eligibility should be made on an anonymous basis by a panel of independent international experts in the field of hyperandrogenism that would in each case issue a recommendation on eligibility for the sport concerned. The investigation of a particular case should be conducted under strict confidentiality (IOC Regulations on female hyperandrogenism -Olympic.org)

CAH Child Reared as a Boy

If the affected child has been reared as a boy due to extreme virilization and brought to the physician for "undescended testes" or for hypospadias, it may be problematic to change the sex of rearing to female. Counselling the parents, with the help of a child psychologist, explaining the true nature of the condition as well as the difficulties the patient may face in adult life, is very important. Rearing up these 46XX girls as males may be the only recourse available in such patients. They require removal of uterus, tubes and ovaries, doing a urethroplasty and putting testicular implants at post pubertal age along with androgen support (Fig 20).

Fig. 20 - Congenital adrenal hyperplasia reared as a boy with enlargement of the breast with external genitalia fully masculinised

In a study of long-term outcomes in 46XX adult patients with congenital adrenal hyperplasia reared as males, thorough psychosexual assessments in adulthood revealed well established male gender identities compatible with their male gender assignments

at birth. In all patients reared as males, the gender role and behaviour were consistent with male gender identity including sexual intercourse with female partners. [35]

Four XX CAH reared as boys and followed up to adulthood developed a clear male gender identity, and when evaluated for sexual activity, they have reported having erections, libido, orgasms, and sexual attraction to women only. Two of these four patients had satisfactory sexual intercourses when assessed using the International Index of Erectile Function questionnaire.[36]

In the Indian scenario of 173 children diagnosed with CAH at the Pediatric Intersex Clinic at AIIMS, seven children presented late with severe virilization and were reared as males. All of them were assigned male sex with the removal of the female adnexa. Six were treated with male genitoplasty. Appropriate hormonal supplementation was offered after puberty. Social adjustments were good in all, except in one who had a bigender mental makeup.[37]

Gender Identity in CAH

Gender identity in girls with CAH was not related to the degree of genital virilization or age at which genital reconstructive surgery was done. Thus, moderate androgen excess early in development appears to produce a small increase in the risk of atypical gender identity, but this risk cannot be predicted from genital virilization. [38]

Late Management Issues

Sexual Activity. Children who had genital surgery done in childhood may have problems later in sexual life since their sensuality may be reduced by surgery. The possibility that women with CAH have deficient clitoral sensation ab initio cannot be excluded. Restricted introitus due to vaginal surgery and reduced sensitivity and psychological factors may affect the normal sexual life of the individual. But many adjust themselves and have normal sexual

activity and will be able to bear children with proper medication. CAH is a lifelong problem and the transition from childhood to puberty and later adulthood needs proper counselling by a team of persons well versed with the management of this complex problem. They need guidance especially on psychological issues. As they reach adulthood fertility, obesity, metabolic syndromes, cardiovascular risks and bone health are to be monitored.

Adrenal Adenomas

In CAH the continued stimulation of adrenals results not only in hyperplasia but can develop adenomas. These are silent adenomas and incidentally discovered during routine ultrasound examination done for some other reason. (Fig 21).

Fig. 21 - CAH child on treatment had adrenal adenoma incidentally discovered on routine ultrasound examination. Histologically a benign adenoma.

Adrenal incidentaloma in patients with homozygous or heterozygous congenital adrenal hyperplasia has been reported .[39]

The Need for Bilateral Adrenalectomy in CAH

Some patients who were well controlled with drugs slowly develop increased requirements of steroids without any apparent reason. They

have progressive signs of both androgen and glucocorticoid excess as obesity and other signs of hypercortisolism .[40] They have adrenocortical macronodular hyperplasia. They were adrenalectomized because attempts to keep their adrenals suppressed had proven ineffective. Prophylactic adrenalectomy in young children with double null mutations remains experimental.[41]

Gmyrek GA advised laparoscopic bilateral adrenalectomy as the primary mode of management in CAH since he considered that current medical therapy for congenital adrenal hyperplasia (CAH) attributable to a complete 21-hydroxylase deficiency is not optimal. He reported the use of laparoscopic bilateral adrenalectomy as a definitive therapy for this condition and argue that it is superior to conventional medical therapy in selected patient. [42] Daniel F reported prophylactic adrenalectomy in a three year old child with CAH. [43]

One of the non CAH causes of clitoromegaly are tumours arising from the clitoris. Of this neurofibroma of the clitoris is seen more often. (Fig 22)

Fig. 22 - Neurofibroma of clitoris simulating clitoromegaly, showing café au lait spots

Neonatal Diagnosis of CAH by Screening

Measurement of 17-OHP levels on dried blood samples is a simple test done in many developed countries as mass screening programme. The screening process, however, is less reliable among low birthweight or preterm infants, borderline elevations require follow up and are considered normal. It can also be falsely negative if the neonate had

received dexamethasone. The normal level of 17 OHP is less than 100ng/dl. In affected neonates, it is raised to 10000ng/dl.

False positives are present if the 17OHP levels are done within three days of birth and premature children. The gold standard is synacthen test. Salt losing CAH have highest values followed by simple virilising and nonclassic types.

Urinary steroid profiling (USP) can quantify metabolites of all relevant steroids simultaneously in a single analysis and has established clinical applications in the investigation and diagnosis of these disorders. USP is used for the investigation of CAH and DSD. In the review of 432 patients of CAH and DSD, Chan AO showed diagnostic pattern of 21-hydroxylase deficiency (n=21), 5α-reductase 2 deficiency (n=12), 17α-hydroxylase deficiency (n=3), isolated 17,20-lyase deficiency (n=1), 11β-hydroxylase deficiency (n=1) and P450 oxidoreductase deficiency (n=1). He suggested that USP is a useful tool in the investigation and diagnosis of CAH and DSD due to different steroidogenesis defects and should be included as a first-line endocrine investigation in this group of patients.[44]

CAH in Siblings and Prenatal Diagnosis

CAH in siblings occurs if the father and mother are carriers of the recessive gene of CAH. (Fig. 23)

Fig. 23 - CAH in siblings. Note mild puffiness of the face of the elder child due to steroids

Screening is necessary for the second pregnancy. Amniocentesis between 16 to 20 weeks of pregnancy or chorionic villi sampling at 8 to 10 weeks of pregnancy will be diagnostic. Point mutations of the CYP21 gene could be detected after specific PCR amplification of the functional CYP21 gene. Study of the C4-CYP21 gene locus by southern blot analysis and that of the

CYP21gene mutations by PCR simplify the procedures for an early and accurate prenatal diagnosis in the first trimester. [45]

Prenatal Therapy

Possible combinations of factors where prenatal therapy should be considered :

- Families with one affected child (index case) with classic CAH (CYP21A2)
- Known parental heterozygosity for classic CAH (non index case)
- New relationship of a parent of a child with classic CAH if the new partner is known to be a carrier for classic CAH
- Homozygosity or compound heterozygosity for classic CAH of one parent when the other parent is a heterozygous gene carrier for classic CAH

Prenatal therapy has been proposed for preventing the in utero virilization of CAH females. Prenatal genetic diagnosis via amniocentesis or chorionic villus sampling is used to determine the need for continuing fetal therapy (dexamethasone), allowing cessation if the fetus was unaffected. The rationale of treatment involves providing sufficient glucocorticoid levels to the fetus to suppress excessive ACTH stimulation. Dexamethasone was chosen because it crosses the placenta and has a long half-life. The protocol was as follows: treatment was proposed to mothers at risk to have a fetus with a classical form of CAH, and who decided to continue pregnancy whether the fetus was affected or not. Treatment had to be started early (< 8th week of amenorrhoea), i.e. prior to any possible prenatal diagnosis. It is still experimental and not allowed in general population.

Needlessly many mothers who were normal were also subjected to therapy. Dexamethasone administered to mother after diagnosis can reduce the virilization. But the risks to the foetus like increased risk of abortion and neurological impairment of the foetus has to be kept in

mind. Potential risks include congenital malformations such as cardiac septal hypertrophy, hydrometrocolpos and hydrocephalus. Moreover, the effects of abrupt dexamethasone withdrawal on fetal development are unknown. In a different study, approximately one fourth of the women treated throughout pregnancy reported some side-effect (e.g., excessive weight gain, severe cutaneous striae, mood fluctuations and irritability, acne and hirsutism, or oedema). [46]

Newer methods now allow diagnosis earlier in gestation, further shortening the treatment time for unaffected female foetuses who will not develop genital ambiguity. Preimplantation genetic testing permits transfer only of an unaffected female or male foetus. Analysis of maternal cell-free DNA based on quantitative differences in the amount of allele parental DNA permits affected pregnancies to be differentiated from unaffected pregnancies.[47]

It has been known for at least a decade that fetal cells are found in maternal blood. A new highly sensitive real-time PCR was developed to detect an SRY gene sequence in maternal serum in the first trimester (6–11 weeks pregnancy). No false negative results were observed for detection of Fetal DNA in maternal serum very early in pregnancy. This may have clinical implications such as with the management of pregnant women carrying a fetus at risk for congenital adrenal hyperplasia.[48] Given the small number of potentially affected patients being treated, clinical research of prenatal treatment should be conducted only in centers of excellence coordinating treatment protocols with multicenter studies standardized registries.[49]

Psychosocial Aspect and Quality of Life in CAH Patients

The prenatal exposure of the foetus to androgens and cerebral imprinting can alter the body image and there is an increased incidence of homosexuality. However many adjust to female gender and with proper medication have normal sexual activity and fertility. The quality of life in the adult period may be affected by the continued

administration of steroids which may result in obesity, insulin resistance and the need for lifelong surveillance. Overall reduction in height and fertility is present in both sexes with CAH. [50]

In patients with CAH, gender identity disorder is a rare finding. Hormonal control, social, familial, and religious beliefs have an impact on the gender identity of these patients. [51]

Women with salt-losing CAH sometimes encounter a situation where suppression of hyperandrogenism requires a high dose of glucocorticoids, resulting in unacceptable side effects such as obesity, glucose intolerance, and hypertension. In this group, bilateral laparoscopic adrenalectomy has been advocated. Final judgment on this is reserved, but benefits in terms of enhanced fertility have been reported.

Overall survival and quality of life is good provided there is proper compliance of drug intake which is lifelong. Deaths in CAH mainly happens in severe salt losing type of CAH especially during periods of stress and illness due to insufficient replacement of steroids. CAH children manifest normal neuropsychological development. Moreover, despite a tendency toward male gender role behaviour and homoerotic fantasy, most girls with CAH identify as females and exhibit heterosexual preference. Gastaud evaluated long term outcomes in 35 women with CAH and almost all women were satisfied with their gender assignment. However, many had pain in penetrative intercourse, only 17 % had children and in 20 % homosexual inclinations were noted. [52]

CAH is a complex problem. If recognised early and treated by a team well versed with the management and the patient is also compliant with proper drugs intake, they can have normal growth, sexual life and fertility.

Key points

- The commonest cause of 46 XX DSD is congenital adrenal hyperplasia.
- The commonest enzyme deficiency causing 46XX DSD is 21hydroxylase which is necessary for cortisone synthesis.
- Synthetic ACTH stimulation will be diagnostic and will identify other enzyme deficiencies like 3beta hydroxysteroid dehydrogenase deficiency.
- All children need lifelong steroid supplement.
- Most of the clitoromegaly in infancy will normalise with adequate amount of steroids. Slight residual clitoromegaly may be acceptable as the child grows.
- The indications for early surgery are social factors and parental pressures though there is no need for a hurry to do clitoroplasty at a very early age.
- Reduction clitoroplasty is preferable to recession clitoroplasty and corpora preserving clitoroplasty since noncompliance with drugs may result in stump hypertrophy and painful erections in corpora preservation surgery.
- Simple posterior vaginal wall mobilisation vaginoplasty is enough for low confluence common channel.
- For high confluence total urogenital mobilisation and partial urogenital mobilisations have been done. Dissections higher to pubourethral membrane carry the risk of urinary incontinence.
- The vaginoplasty can be planned after the child is 10 years of age.

- With proper medication and surgery, fertility and pregnancy is possible.
- Inspite of the prenatal androgen imprinting of the brain almost all have feminine gender identity and are heterosexual. Small percentage of the above are homosexual and have gender dysphoria.
- It is possible now to make prenatal diagnosis of CAH by chorionic villi sampling and Amniocentesis.
- Maternal hormone administration to reduce virilization of the foetus has not found favour since the complications of high dose steroids to the mother and to the developing foetus precludes its routine use unless the team is well trained to manage the issues.
- All will need proper social and psychological support to address the issues of gender identity and gender role, preference of sexual partners and sexual and reproductive issues.

References

1) Perrin C. White Phyllis W. Speiser: Congenital Adrenal Hyperplasia due to 21-Hydroxylase Deficiency Endocrine Reviews, Volume 21, Issue 3, 1 June 2000, Pages 245–291.

2) Rink RC, Adams MC, Misseri R. A new classification for genital ambiguity and urogenital sinus anomalies. BJU Int 2005;95: 638–42.

3) Merke DP, Chrousos GP, Eisenhofer G, Weise M, Keil MF, Rogol AD, Van Wyk JJ, Bornstein SR. Adrenomedullary dysplasia and hypofunction in patients with classic 21-hydroxylase deficiency.N Engl J Med. 2000 Nov 9;343(19):1362-8.

4) Charmandari E, Eisenhofer G, Mehlinger SL, Carlson A, Wesley R, Keil MF, Chrousos GP, New MI, Merke DP. Adrenomedullary function may predict phenotype and genotype in classic 21-hydroxylase deficiency. J Clin

Endocrinol Metab. 2002 Jul;87(7):3031-7.

5) Dewailly Dvantyghem-HaudiquetMC Sainsard Buvat1986 Clinical and biological phenotypes in late-onset 21-hydroxylase deficiency. J Clin Endocrinol Metab. 1986 Aug;63(2):418-23.

6) Phyllis W. Speiser Congenital Adrenal Hyperplasia Due to Steroid 21-Hydroxylase Deficiency: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab. 2010 Sep; 95(9): 4133–4160.

7) Andrew Calabria, MD, Congenital Adrenal Hyperplasia Caused by 11Beta-Hydroxylase Perelman School of Medicine at The University of Pennsylvania MSD manual professional version.

8) Simard J, Moisan AM, Morel Y "Congenital adrenal hyperplasia due to 3beta-hydroxysteroid dehydrogenase/Delta (5) - Delta (4) isomerase deficiency". Semin. Reprod. Med. (August 2002).

9) Levran, David; Ben-Shlomo, Izhar; Pariente, Clara; Dor, Jehoshua; Mashiach, Shlomo; Weissman, Ariel (2003). "Familial Partial 17,20-Desmolase and 17α-Hydroxylase Deficiency Presenting as Infertility". Journal of Assisted Reproduction and Genetics. 20 (1): 21–8.

10) Idkowiak J1, Cragun D2, Hopkin RJ3, Arlt Cytochrome P450 Oxidoreductase Deficiency GeneReviews. Seattle (WA): University of Washington, Seattle; 1993-2019. 2005 Sep 8 updated 2017 Aug 3.

11) Arlt, W., Walker, E.A., Draper, N., Ivison, H.E., Ride, J.P., Hammer, F., Chalder, S.M., Borucka - Mankiewicz, M., Hauffa, B.P., Malunowicz, E.M., Stewart, P.M. & Shackleton, C.H. (2004) Congenital adrenal hyperplasia caused by mutant P450 oxidoreductase and human androgen synthesis: analytical study. Lancet, 363, 2128–2135.

12) Shackleton C1, Marcos J, Arlt W, Hauffa BP Prenatal diagnosis of P450 oxidoreductase deficiency (ORD): a disorder causing low pregnancy estriol, maternal and fetal virilization, and the Antley-Bixler syndrome phenotype. Am J Med Genet A. 2004 Aug 30;129A(2):105-12.

13) Idkowiak J1, Cragun D2, Hopkin RJ3, Arlt Cytochrome P450 Oxidoreductase Deficiency GeneReviews® [Internet]. Seattle (WA):

University of Washington, Seattle; 1993-2019. 2005 Sep 8 updated 2017 Aug 3.

14) Chan Jong Kim, MD Congenital lipoid adrenal hyperplasia Ann Pediatr Endocrinol Metab. 2014 Dec; 19(4): 179–183.

15) Sandison AT. A form of lipoidosis of the adrenal cortex in an infant. Arch Dis Child 1955;30:538-541P.

16) Kirkland RT, Kirkland JL, Johnson CM, Horning MG, Librik L, Clayton GW. Congenital lipoid adrenal hyperplasia in an eight-year-old phenotypic female. J Clin Endocrinol Metab 1973;36:488-496.

17) Gucev ZS, Tee MK, Chitayat D, Wherrett DK, Miller WL. Distinguishing deficiencies in the steroidogenic acute regulatory protein and the cholesterol side chain cleavage enzyme causing neonatal adrenal failure. J Pediatr. 2013;162:819–822.

18) Himangshu S. Bose Seiji Sato Javier Aisenberg Stavit A. Shalev Nobutake Matsuo Walter L. Miller: Mutations in the Steroidogenic Acute Regulatory Protein (StAR) in Six Patients with Congenital Lipoid Adrenal The Journal of Clinical Endocrinology & Metabolism, Volume 85, Issue 10, 1 October 2000, Pages 3636–3639.

19) Pang, S., Hotchkiss, J., Drash, A.L., Levine, L.S. & New, M.I. (1977) Microfilter paper method for 17 alpha hydroxyprogesterone radioimmunoassay: its application for rapid screening for congenital adrenal hyperplasia. Journal of Clinical Endocrinology and Metabolism, 45, 1003–1008.

20) Clayton, P.E., Miller, W.L., Oberfield, S.E., Ritzen, E.M., Sippell, W.G. & Speiser, P.W. (2002) Consensus statement on 21hydroxylase deficiency from the European Society for Paediatric Endocrinology and the Lawson Wilkins Pediatric Endocrine Society. Hormone Research, 58, 188–195.

21) Merke, D.P. & Bornstein, S.R. (2005) Congenital adrenal hyperplasia. Lancet, 365, 2125–2136.

22) Phyllis W. Speiser, et al Congenital Adrenal Hyperplasia Due to

Steroid21-Hydroxylase Deficiency: An EndocrineSociety Clinical Practice Guideline (J Clin Endocrinol. Metab 103: 4043–4088, 2018).

23) Baskin LS1 Restoring normal anatomy in female patients with atypical genitalia. Semin Perinatol. 2017 Jun;41(4):227-231.

24) Pippi Salle JL1, Braga LP, Macedo N, Rosito N, Bagli D. Corporeal sparing dismembered clitoroplasty: an alternative technique for feminizing genitoplasty J Urol. 2007 Oct;178(4 Pt 2):1796-800.

25) Baskin LS1, Erol A, Li YW, Liu WH, Kurzrock E, Cunha GR Anatomical studies of the human clitoris. J Urol. 1999 Sep;162(3 Pt 2):1015-20.

26) Baskin LS Anatomical studies of the female genitalia: surgical reconstructive implications. J Pediatr Endocrinol Metab. 2004 Apr;17(4):581-7.

27) 1 Peña A. Total urogenital mobilization – an easier way to repair cloacas. J Paediatr Surg 1997; 32: 263–7 .

28) Jesus VM1, Buriti F1, Lessa R1, Toralles MB1, Oliveira LB1, Barroso U Jr2. Total urogenital sinus mobilization for ambiguous genitalia. J Pediatr Surg. 2018 Apr;53(4):808-812.

29) Hamza AF1, Soliman HA, Abdel Hay SA, Kabesh AA, Elbehery MM.Total urogenital sinus mobilization in the repair of cloacal anomalies and congenital adrenal hyperplasia. J Pediatr Surg. 2001 Nov;36(11):1656-8.

30) Stites J1, Bernabé KJ1, Galan D1, Felsen D1, Poppas DP2 Urinary continence outcomes following vaginoplasty in patients with congenital adrenal hyperplasia J Pediatr Urol. 2017 Feb;13(1):38 Nov 22.

31) Rink RC, Metcalfe PD, Kaefer MA, Casale AJ, Meldrum KK, Cain MP. Partial urogenital mobilization: A limited proximal dissection. J Paediatr Urol 2006; 2: 351–6.

32) Crouch NS[l], Liao LM, Woodhouse CR, Conway GS, Creighton SM Sexual function and genital sensitivity following feminizing genitoplasty for congenital adrenal hyperplasia.J Urol. 2008 Feb;179(2):634-8.

33) Snyder HM, Retik AB, Bauer SB, Colodny AH. Feminizing genitoplasty: a synthesis. J Urol. 1983;129:1024-6.

34) Lo JC[1], Grumbach MM Pregnancy outcomes in women with congenital virilizing adrenal hyperplasia. Endocrinol Metab Clin North Am. 2001 Mar;30(1):207-29.

35) Khattab A1 et al Long term outcomes in 46, XX adult patients with congenital adrenal hyperplasia reared as males. J Steroid Biochem Mol Biol. 2017 Jan;165 .

36) Apóstolos RAC et al Gender Identity and Sexual Function in 46 XX Patients with Congenital Adrenal Hyperplasia Raised as Males. Arch Sex Behav. 2018 Nov;47(8):2491-2496.

37) Sharma S1, Gupta DK. Male genitoplasty for 46 XX congenital adrenal hyperplasia patients presenting late and reared as males. Indian J Endocrinol Metab. 2012 Nov;16(6):935.

38) Berenbaum SA, Bailey JM. Effects on the gender identity of prenatal androgens and genital appearance: evidence from girls with congenital adrenal hyperplasia.J Clin Endocrinol Metab. 2003 Mar;88(3):1102.

39) S Jaresch E Kornely H K Kley R Schlaghecke Adrenal incidentaloma and patients with homozygous or heterozygous congenital adrenal hyperplasia The Journal of Clinical Endocrinology & Metabolism, Volume 74, Issue 3, 1 March 1992, Pages 685–689.

40) Doppman JL[1], Adrenocorticotropin-independent macronodular adrenal hyperplasia : an uncommon cause of primary adrenal hypercortisolism. A Radiology. 2000 Sep;216(3):797-802.

41) Judson J. Van Wyk E. Martin RitzenThe Role of Bilateral Adrenalectomy in the Treatment of Congenital Adrenal Hyperplasia The Journal of Clinical Endocrinology & Metabolism, Volume 88, Issue 7, 1 July 2003, Pages 2993–2998.

42) Gmyrek GA, New MI, Sosa RE, Poppas DP Bilateral laparoscopic

adrenalectomy as a treatment for classic congenital adrenal hyperplasia attributable to 21-hydroxylase deficiency. Pediatrics. 2002 Feb;109(2): E28.

43) Daniel F. Gunther Timothy P. Bukowski E. Martin Ritzén Anna WedellJudson J. Van Wyk Prophylactic Adrenalectomy of a Three-Year-Old Girl with Congenital Adrenal Hyperplasia: Pre- and Postoperative Studies The Journal of Clinical Endocrinology & Metabolism, Volume 82, Issue 10, 1 October 1997, Pages 3324–3327.

44) Chan AO1, Shek CC.Urinary steroid profiling in the diagnosis of congenital adrenal hyperplasia and disorders of sex development: experience of a urinary steroid referral center in Hong Kong. Clin Biochem. 2013 Mar;46(4-5):327-34.

45) Bradley JF1, Baker D, Schwartz ID, Rothberg PG The Importance of Heteroduplexes in Interpreting the Results of c: Application to the Analysis of Mutations in the Steroid 21-Hydroxylase Gene in a Case of Congenital Adrenal Hyperplasia. Mol Diagn. 1998 Jun;3(2):119-123.

46) Lajic S1, Bui TH, Holst M, Ritzén M, Wedell A Prenatal diagnosis and treatment of adrenogenital syndrome. Prevent virilization of female foetuses. Lakartidningen. 1997 Dec 10;94(50):4781-6.

47) Simpson JL1, Rechitsky S2: Prenatal genetic testing and treatment for congenital adrenal hyperplasia. Fertil Steril. 2019 Jan;111(1):21-23.

48) Guibert J, Benachi A, Grebille AG, Ernault P, Zorn JR, Costa JM. Kinetics of SRY gene appearance in maternal serum: detection by real time PCR in early pregnancy after assisted reproductive technique Hum Reprod. 2003 Aug;18(8):1733-6.

49) Phyllis W. Speiser Congenital Adrenal Hyperplasia due to Steroid 21-Hydroxylase Deficiency: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab. 2010 Sep; 95(9): 4133–4160.

50) Otten BJ1, Stikkelbroeck MM, Claahsen-van der Grinten HL, Hermus AR.J Clin Endocrinol Metab. 2007 Apr;92(4):1391-6.

51) Razzaghy-Azar M1,2, Karimi S1, Shirazi E1,3Endocr Dev. 2005;8:54-

66. Gender Identity in Patients with Congenital Adrenal Hyperplasia.Puberty and fertility in congenital adrenal hyperplasia.Int J Endocrinol Metab. 2017 Jul 30;15(3).

52) Gastaud F1, Bouvattier C, Duranteau L, Brauner R, Thibaud E, Kutten F, Bougnères P Impaired sexual and reproductive outcomes in women with classical forms of congenital adrenal hyperplasia. J Clin Endocrinol Metab. 2007 Apr;92(4):1391-6.

Chapter 4

46 XY DSD 5ά Reductase Deficiency

5ά Reductase Deficiency

5ά reductase deficiency was initially called “pseudovaginal perineoscrotal hypospadias”.[1] One of the first people to study this unusual condition was Dr. Julianne Imperato, a Cornell endocrinologist. She travelled to the remote part of the Dominican Republic in the 1970s because of strange rumours about girls turning into boys. 5ά - reductase deficiency is common in Papua New Guinea and some middle east countries. These individuals have been referred to by different names in their regions, including guavadoces, translated to mean "penis at age twelve," and machihembras, which means "first a woman, then a man." In Papua New Guinea, the locals called these individuals turnims, meaning "expected to become men."

When Dr. Imperato investigated the Guavadoces, she discovered the reason they don’t have male genitalia at birth is because they are deficient in an enzyme called 5-α-reductase, which normally converts testosterone into dihydro testosterone. So, they appear female when they are born, but around puberty, when they get another surge of testosterone, they masculinise with an increase in the size of penis and the testis increases in volume and descends. Apart from having slightly undersized penis, everything works and the Guavadoces normally live out their lives as men, albeit with wispy beards and small prostates.[2]

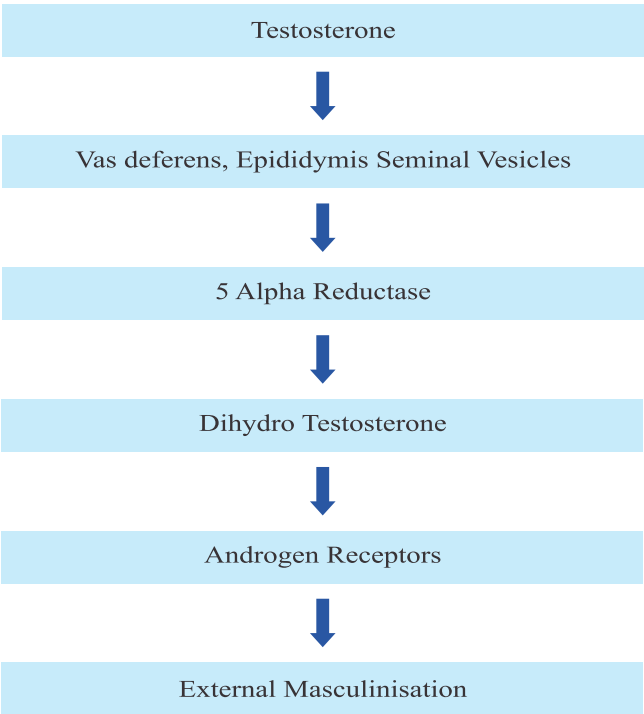


Fig. 1 - Role of 5 Alpha Reductase

Testosterone (T) secreted by the fetal testes is converted to dihydrotestosterone (DHT) by 5α-reductase, an enzyme in the primordia of the external genitalia (Fig1). Dihydrotestosterone is necessary for the genital tubercle to differentiate into the penis and the labioscrotal folds to fuse. Male external genitalia are complete by 12–16 weeks. Dihydrotestosterone also stimulates the formation of the prostate and Cowper's glands. Testosterone alone cannot accomplish these steps, although it can produce virilization at puberty. At puberty, T and not DHT is the essential androgen for growth of the male external genitalia and the emergence of male secondary sex characteristics. Thus, masculinising puberty ensues, the phallus markedly enlarges, the testes descend into the labioscrotal folds, the

beard grows, the voice deepens, and a masculine habitus develops. It is also postulated that 5a-reductase type 1 surge at the time of puberty being responsible for virilization in type 2-deficient subjects during puberty. 5α-Reductase activity has been demonstrated in tissue of the urogenital sinus, urogenital swellings, and urogenital tubercle, but not in that of the Wolffian duct analage.

In the absence of 5α -reductase enzyme, testosterone (T) is not converted to the biologically active form, dihydrotestosterone (DHT). In the absence of DHT, the infant's external genitalia appear to be ambiguous where the genitalia is grossly under virilized with small penis simulating a clitoris with labio scrotal folds unfused with rudimentary vagina (Fig 2). The karyotype is XY. It is inherited as an autosomal recessive gene. Since the Sertoli cells are unaffected, they have Mullerian inhibiting substance and hence uterus and appendages are not present.

Genetics of 5α Reductase Deficiency

The 5α-reductase type 2 gene (gene symbol SRD5A2) was cloned and shown to contain five exons and four introns. The gene was localized to chromosome 2 band p23 by somatic cell hybrid mapping and chromosomal in situ hybridization. Molecular analysis of the SRD5A2 gene resulted in the identification of 18 mutations in 11 homozygotes, 6 compound heterozygotes, and 4 inferred compound heterozygotes from 23 families with 5a-reductase deficiency. [3]

Clinical Description

Patients present at birth with characteristics of dihydrotestosterone (DHT) deficiency, such as under virilized penis, clitoris-like phallus and unfused labioscrotal folds with hypospadias (Fig 2). They may have a blind vaginal pouch and will have bilateral gonads

Fig. 2 - 46 XY : Feminised appearance of 5 a reductase deficiency. Labio scrotal folds are unfused with testes descended with a diminutive penis.

which are testes. If there is unilateral gonad one has to keep in mind the possibility of mixed gonadal dysgenesis or true hermaphroditism (ovotesticular DSD). The child may present with minimal under virilization with normal male anatomy except for isolated micropenis or hypospadias. (Fig 3)

Fig. 3 - Isolated micropenis with hypospadias

However, during puberty, scarce facial and body hair (with normal sebum production) and virilization without breast development are observed. There will be an increase in the penile size as well as the muscle mass and a masculine voice. This is often accompanied by gender identity change from female to male. Pubertal virilization occurs in 5α-RD-2, most likely caused by the direct action of testosterone on the phallus and also because at this age there is increased production of 5α-RD-1, which converts part of the pubertal testosterone into dihydrotestosterone.[4] Gender assignment in these patients has been debated because initially, the external appearance is that of a girl child with major virilization occurring at puberty.

Prenatal Diagnosis

Prenatal diagnosis is not possible for 5-alpha-reductase type 2 deficiency (5-ARD) and diagnosis is usually made in the newborn period when the infant presents with ambiguous genitalia. No risk

factors or clinical markers in pregnancy are known. Genital ambiguity is occasionally diagnosed prenatally when an infant who is demonstrated by amniocentesis or chorionic villus sampling to have XY karyotype fails to have a demonstrable penis on ultrasonography.[4]

The risk of gonadal tumours in individuals with SRD5A2 is quite low. Prostate diseases (prostate cancer, benign prostate hyperplasia) have not been reported in affected males. Most men are infertile due to azoospermia or oligospermia associated with undescended testes.

Diagnostic Methods

Diagnostic criteria include genital ambiguity, a family history of DSD, and genital-karyotype discordance. Biochemical findings reveal an increase in the T-to-DHT ratio after HCG stimulation and normal T and anti-Müllerian hormone concentration. There is a high ratio of serum T to DHT. Normal patients respond with ratios from 8-16, while patients with 5-alpha-reductase-2 deficiency exhibit T/DHT ratios from 35-84. During the first 60 days of life, infants experience a surge of LH that obviates the need to carry out HCG stimulation,

Diagnosis also relies on a 5-alpha-reductase activity assessment using the urinary steroid profiling (ratio of androsterone to etiocholanolone and 5-alpha-tetrahydrocortisol / tetrahydrocortisol and 5-alpha-tetra hydrocorticosterone to tetra hydrocorticosterone) below the lower limit of normal. The diagnosis is confirmed by genetic screening. Decreased 5 alpha-reductase activity was demonstrated in fibroblasts cultured from genital skin.[5] 5α-Reductase activity is higher in some tissues than in others, which is why it is preferable to assay cells derived from genital tissue (e.g. foreskin). There is, however, considerable variability in 5α-reductase activity among control genital tissue, with near-overlap between controls and persons recognized on other grounds to have 5α-reductase deficiency. Thus 5α-reductase activity in cultured genital fibroblasts excludes the diagnosis of 5α-reductase deficiency, but the absence of 5α-reductase

offers less confidence in confirming this diagnosis. Phallic growth in response to DHT cream treatment could be an indirect confirmation of 5 alpha reductase deficiency.[6]

In infancy, the T/DHT ratio, assessed by suitable assay methods and evaluated by age-appropriate reference values, seems to be able to select newborns affected by 5alpha-reductase-2 deficiency. Molecular analysis of the SRD5A2 gene should be warranted in newborns with abnormal ratios before sex assignment. [7]

Differential Diagnosis

The phenotypes of male pseudohermaphrodites with 5α-RD2, 17β hydroxysteroid dehydrogenase deficiency, Partial androgen insensitivity syndrome (PAIS) and Leydig cell hypoplasia are similar.

The differential diagnosis of the androgen insensitivity syndrome (AIS) and 5α-RD2 should be made early because individuals with complete AIS are usually raised as females, and those with 5α-RD2 as males when the diagnosis is made during infancy. HCG stimulation and measurement of testosterone and DHT will help to differentiate under virilization due to different conditions.

Multiple protocols for HCG stimulation test , both short-term and long-term, have been established with varying sensitivities. Three common protocols include the following: (1) 1500 IU intramuscularly (IM) on days 1, 3, and 5 (short test) (2) 1500 IU IM every other day for 7 injections (prolonged test); or (3) 5000 IU/m2 IM as a single dose. Baseline laboratory results are established prior to HCG administration and stimulated laboratory studies are drawn 24 hours after the last dose for the multi-day tests, or 72 hours after the single-dose test. [8]

If the baseline testosterone level itself is low and does not show an increase in HCG stimulation it indicates Leydig cell hypoplasia. In

5α-RD2 deficiency, the elevated serum testosterone-to-DHT ratio (T/DHT) is the hallmark of 5-alpha-reductase type 2 deficiency. The 17β- HSD3 deficiency is caused by impaired testicular conversion of androstenedione (AT) to T. Individuals have an increased ratio of AT to T in baseline samples or following HCG stimulation. In PAIS, the testosterone and DHT levels are either normal or increased after HCG stimulation. (Fig 4)

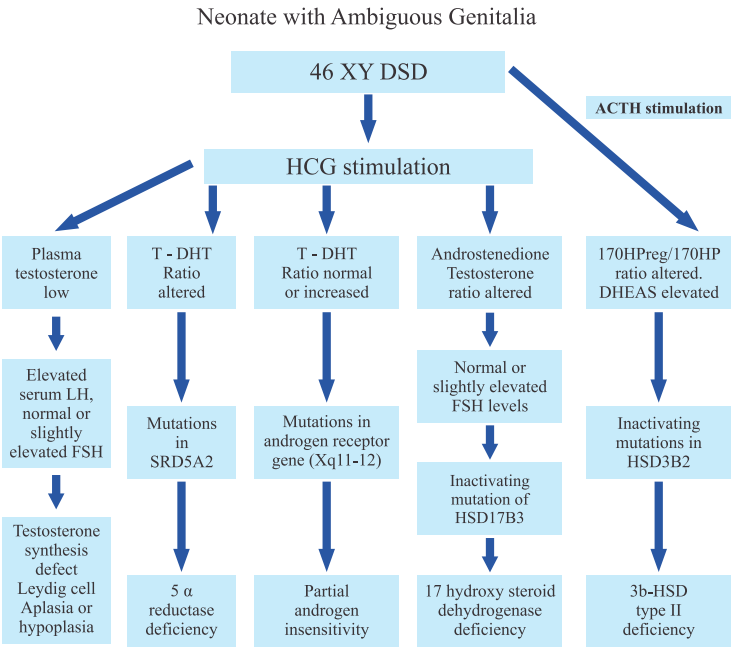


Fig. 4 - Flow Chart on Differential Diagnosis of XY DSD

Genetic Diagnosis

Molecular analysis of the SRD5A2 should be recommended in newborns with an abnormal ratio before gender assignment and may be employed as a tool for the early and precise diagnosis of patients

with 46XY DSD. Mutation analysis of the 5-alpha-reductase type 2 gene (SRD5A2) can be done. Testing is performed via exon array comparative genomic hybridization (CGH) or by direct gene sequencing. Utility of this testing includes confirmation of a clinical diagnosis; differentiation of 5-alpha-reductase type 2 deficiency from other causes of 46XY DSD.

Clinically, phallic growth in response to DHT cream treatment could be an indirect confirmation of 5 alpha-reductase deficiency.

Imaging Studies - Ultrasonography

Although not diagnostic for 5-alpha-reductase type 2 deficiency, ultrasonography can verify the location of the testes and other Wolffian structures as well as the absence of Mullerian structures.

Management of children reared as boys

Neonates and children who are born with under virilization and diagnosed as 5 alpha reductase deficiency by laboratory and genetic investigations should be reared as male children. [9]

They virilize at puberty. After confirmation of the diagnosis by molecular analysis of the SRD5A2 gene, a satisfactory change to a male phenotype can be achieved by hormone treatment preceding surgery.[10] They may require androgen supplementation for virilization. The surface application of DHT cream may help in an increase in penile size. Administration of testosterone during the prepubertal period helps in the development of penis. In patients with 5-alpha-reductase deficiency, limited data suggest that high-dose testosterone may increase penile shaft length and circumference, increase erectile potency and ejaculatory volume, increase facial hair and muscularity, and improve a sense of well-being. Surgical repair of hypospadias (chordee correction, orchidopexy, and urethral reconstruction) should be performed at 6-18 months of age.

Androgens that do not require 5α-reductase, such as 19 nortestosterone, also can be given.

Sexual Activity and Fertility

Overall satisfaction with sexual life was found to be satisfactory in individuals with a small penis, from a study of those who had traumatic partial penile amputation in childhood. [11,12]

In a different questionnaire to married women on sexual satisfaction and penile size, it was found that the penile length had not made any difference though the girth did make some difference.[13]

Paternity by intrauterine insemination is feasible in men with 5α-reductase-2 deficiency, affirming their full reproductive potential and providing further support for raising them as males. [14]

Management of Children Reared as Girls

In older children who have been raised as females, management includes surgical correction of the external genitalia (vaginal opening into the perineum with early separation of the vagina and urethra), early removal of gonads to prevent masculinization before puberty, clitoral reduction (in severe masculinization) and cyclic hormonal therapy at puberty for development of secondary sexual characteristics. Such persons require removal of the testes to prevent further virilization and to reduce the risk of tumors.

Many have a rudimentary vagina and periodic dilatation will make it acceptable for sexual activity without major reconstructive procedures. Treatment must simulate a normal puberty pattern, and low to normal estrogen doses, taking into account the height, should be administered at the age of expected puberty (10 – 12 years old).

After complete breast development, adult estrogen doses are maintained continuously. Progesterone replacement is not necessary

because these patients do not have a uterus. Estrogen should be continued for feminisation and prevent osteoporosis and tailor the dose to prevent untoward effects of estrogen.

In those in whom there is no vagina, vaginal substitution in the form of colovaginoplasty can be done. Individuals with 5alpha-reductase-2 deficiency (5alpha-RD-2) and 17 beta-hydroxysteroid dehydrogenase-3 deficiency (17beta-HSD-3) are often raised as girls. Over the past number of years, this policy has been challenged because many individuals with these conditions develop a male gender identity and make a gender role change after puberty. However, an estimation of the prevalence of gender role changes, based on the current literature, shows that gender role changes occur frequently, but not invariably. Gender role changes were reported in 56-63% of cases with 5alpha-RD-2 and 39-64% of cases with 17beta-HSD-3 who were raised as girls. The changes were usually made in adolescence and early adulthood. In these two syndromes, the degree of external genital masculinization at birth does not seem to be related to gender role changes in a systematic way.[15]

5α-Reductase deficiency should be investigated in elite young female athletes with primary amenorrhea and high male T levels detected during antidoping programs to identify undiagnosed XY DSD. [16]

Case Series

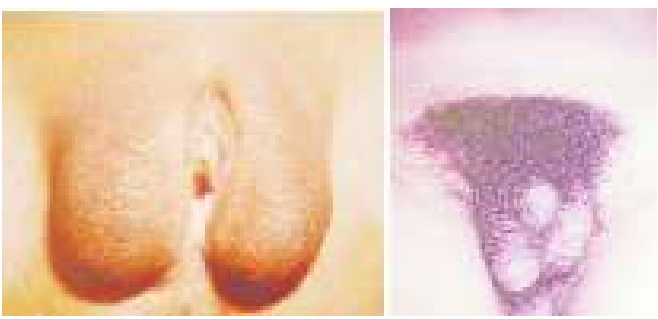
Case 1

A nine-year-old XY DSD child reared as a girl came with the complaint of swelling in labia on both sides. Clinically the unfused labia had gonads with small rudimentary penis. A blind vaginal pouch was present. Karyotype was XY and the ultrasound abdomen showed no Mullerian structures. The parents wanted her to be a girl since her gender role was that of the girl child and the child's preference was also that of a girl. The testicular gonads were removed and feminising genitoplasty was done.

Case 1 - 5 Alpha reductase deficiency reared as a girl, feminising genitoplasty, estrogen induced breast development

At 12 years of age, she had estrogen administration for breast development. At the age of 24 years, she got married to a man who had lost his first wife and had two children. Post married life, she needed enlargement of the vaginal orifice. Subsequent sexual life was normal. Follow up after 25 years showed that there was no sexual dysphoria and she was happy with the female gender.

Case 2



Case 2 - 5 Alpha reductase deficiency reared as a boy who had a small penis, showing reasonable penile growth as he attained puberty

Her brother who had similar external features was reared as a boy. At birth, he had both gonads in scrotum with a small sized penis. Karyotype was XY and the ultrasound abdomen did not show Mullerian structures. He was reared as a male child. He underwent

hypospadias repair and at the age of 14 years he had normal pubic hair and the penile growth was adequate for his age. At 28 years, marriage was proposed but he was diffident because of the slightly undersized penis. He had counselling with an andrologist and got married. At the age of 38 years, he was having a normal sexual life but was azoospermic.

Case 3 - 5 Alpha reductase deficiency reared as a girl late presentation with virilization of external genitalia

Case 3

15-year-old reared as a girl came first in all the sports events in the school and national sports events for women. She wanted to compete in the Asian Olympics. Clinically she had well developed penis with testis in the labia. She had a 3 cm blind vagina. After examination and investigations, she was told that she is a male and asked her preference for gender identity. She and her parents wanted feminization of external genitalia and was surgically corrected as a girl child.

Key points in 5 Alpha Reductase Deficiency

- 5 alpha reductase deficiency which results in DHT deficiency is caused by an autosomal recessive gene in XY individuals and

results in feminisation of external genitalia with an underdeveloped penis, bifid scrotum, rudimentary vagina with the absence of Mullerian structures and the gonads being testes.

- Diagnosis is made based on an increased T/DHT ratio in basal and hCG-stimulation conditions. Measurement of urinary metabolites of testosterone and DHT can be used to establish the diagnosis. Mutation analysis of the 5-alpha-reductase type 2 gene (SRD5A2) is confirmatory. The presence of 5 alpha reductase in genital tissues excludes its deficiency.

- Differential diagnosis includes Leydig cell hypoplasia, 17hydroxysteroid dehydrogenase deficiency, and partial androgen insensitivity and can be differentiated by HCG stimulation test and measuring T and DHT and other metabolites.

- Once diagnosed, it is preferable to rear them as males even in marked under virilization since they will virilize at puberty,

- Those who have been reared as males will need reconstructive procedures of the external genitalia and testosterone supplement at the time of puberty. The surface application of DHT cream can increase the penile size.

- The average penile size is smaller than controls but does not preclude sexual activity.

- Fertility is still possible in spite of the poor quality of semen and diminished sperm count.

- Those reared as girls need feminisation of external genitalia. They need estrogen supplementation for breast development and has to be continued later, the rudimentary vagina is adequate in most cases for normal sexual activity and can be increased in size and depth by periodical dilatations with moulds. Rarely do they need vaginoplasty.

References

1) Walsh PC, Madden JD, Harrod MJ, Goldstein JL, MacDonald PC, Wilson JD Familial incomplete male pseudohermaphroditism, type 2. Decreased dihydrotestosterone formation in pseudovaginal perineoscrotal hypospadias. 'N Engl J Med. 1974 Oct 31;291(18):944-9.

2) Imperato-McGinley J, Miller M, Wilson JD, Peterson RE, Shackleton C, Gajdusek DC. A cluster of male pseudohermaphrodites with 5 alpha-reductase deficiency in Papua New Guinea. Clin Endocrinol (Oxf). 1991 Apr;34(4):293-8.

3) Anice E. Thigpen et al Molecular Genetics of Steroid 5a-Reductase 2 Deficiency J. Clin. Invest Volume 90, September 1992, 799-809.

4) Wilson JD, Griffin JE, Russell DW. Steroid 5 alpha-reductase 2 deficiency. Endocr Rev. 1993 Oct;14(5):577-93. Review.

5) Anice E. Thigpen, Richard 1. Silver,t Joseph M. Guileyardo, I M. Linette Casey," John D. McConnell,t and David W. Russell Tissue Distribution and Ontogeny of Steroid 5a-Reductase Isozyme Expression The American Society for Clinical Investigation, Volume 92, August 1993, 903-910.*

6) I Odame, M D Donaldson, A M Wallace, W CochranEarly Diagnosis and management of 5 alpha-reductase deficiency. Arch Dis Child. 1992 Jun; 67(6): 720–723.

7) Bertelloni S, Scaramuzzo RT, Parrini D, Baldinotti F, Tumini S, Ghirri P. Early diagnosis of 5alpha-reductase deficiency in newborns. Sex Dev. 2007. 1(3):147-51.

8) Kulkarni KP, Panigrahi I, Das R, Kaur S, Marwaha RK. Pediatric disorders of sex development. Indian J Pediatr. 2009 Sep;76(9):956-8 .

9) Cheon CK1 Practical approach to steroid 5alpha-reductase type 2 deficiency. Eur J Pediatr. 2011 Jan;170(1): 1-8. Epub 2010 Mar 28.

10) Walter KN1, Kienzle FB, et al Difficulties in diagnosis and treatment of 5alpha-reductase type 2 deficiency in a newborn with 46, XY DSD. Horm Res

Paediatr. 2010;74(1):67-71. Epub 2010 Apr 16.

11) Reilly JM1, Woodhouse CR. Small penis and the male sexual role. J Urol. 1989 Aug;142(2 Pt 2):569-71; discussion 572.

12) Ochoa B. Trauma of the external genitalia in children: amputation of the penis and emasculation. J Urol. 1998 Sep;160(3 Pt 2):1116-9; discussion 1137.

13) Penis size: Survey of female perceptions of sexual satisfaction. BMC Womens Health. Published online 2001 Jun 8.

14) Melissa D. Katz, M.D., Isaac Kligman, M.D.Paternity by Intrauterine Insemination with Sperm from a Man with 5α-Reductase-2 Deficiency N Engl J Med 1997; 336:994-998April 3, 1997.

15) Cohen-Kettenis PT. Gender change in 46, XY persons with 5alpha-reductase-2 deficiency and 17beta-hydroxysteroid dehydrogenase-3 deficiency. Arch Sex Behav. 2005;34:399–410. (PubMed)

16) Fénichel P1, Paris F, et al. Molecular diagnosis of 5α-reductase deficiency in 4 elite young female athletes through hormonal screening for hyperandrogenism. J Clin Endocrinol Metab. 2013 Jun;98(6Z).

Chapter 5

Androgen Insensitivity Syndrome

Androgen insensitivity syndrome (AIS) is characterised by the female phenotype in an XY individual due to androgen resistance as a result of mutations in the X linked androgen receptor. AIS is caused by one of the multiple existing defects that affect either the quantity, binding affinity, or function of the androgen receptors or downstream signalling pathways. The presence of androgen receptors is essential to mediate the action of both testosterone and dihydrotestosterone. It is present in all tissues except the spleen. It is an X linked recessive disorder. The gene mutations result in the failure of the receptors to bind the androgens and they have qualitative abnormalities. The receptors are present in the cell nucleus and cytoplasm of all genital and non genital tissues. AIS has an incidence of 1:20000 to 60000. AIS represents approximately half of all 46XY DSD.

History

In 1950 Lawson Wilkins tried administration of methyltestosterone to a 46XY female which did not work and is the first documented demonstration of AIS[1]. In 1970 Mary F. Lyon and Susan Hawkes reported that a gene on the X chromosome caused complete insensitivity to androgens in mice.[2] In 1981 Barbara Migeon et al. narrowed down the locus of the human androgen receptor gene (or a factor controlling the androgen receptor gene) to somewhere between Xq11 and Xq13.[3] In1988 Terry Brown et al. reported the first mutations proven to cause AIS and in 1989 he reported the exact locus

of the AR gene (Xq11-Xq12)[4] and Dennis Lubahn et al. published its intron-exon boundaries.[5]

John Morris reviewed the clinical features of 82 patients and observed that the patients were women and girls with bilateral testes that seemed to produce oestrogen like hormones. Morris coined the term "testicular feminisation syndrome".[6] When these women were treated with methyltestosterone, it did not have any effect and urinary 17ketosteroids were normal. So, a conclusion was drawn that it is androgen receptor deficiency and was coined Androgen insensitivity syndrome. Thus, androgen insensitivity syndrome can be defined as a disorder resulting from complete or partial resistance to the biological actions of androgens in an XY individuals with normal testis determination and production of age-appropriate androgen concentrations.[7]

Types of AIS

Complete androgen insensitivity syndrome (CAIS) is a condition where the phenotype of the child or older infants are absolutely feminine. When it is partially virilized it is called Partial androgen insensitivity syndrome (PAIS) and when it is mild form in boys or adults with gynaecomastia or infertility it is called Mild androgen insensitivity syndrome. (MAIS) The phenotypic abnormalities range from complete female appearance in CAIS to complete virilization and male appearance with azoospermia or gynecomastia in MAIS. Clinical presentation is related to the severity of the defect.

Androgens are important steroid hormones for expression of the male phenotype. They are necessary for male sex differentiation and spermatogenesis. The two most important androgens in this respect are testosterone and dihydrotestosterone. Each androgen has its specific role during male sexual differentiation, testosterone is involved in the development and differentiation of Wolffian duct

derived structures, whereas dihydrotestosterone is necessary for normal external genitalia masculinisation. The actions of androgens are mediated by the androgen receptor. The presence of androgen receptors is essential to mediate the action of testosterone and DHT in the target tissues.

Genetics of AIS

The androgen receptor gene is located on the X-chromosome at Xq11-12 and codes for a protein with a molecular mass of approximately 110 kDa. Only one androgen receptor cDNA has been identified so far, despite two different ligands. It is generally accepted that defects in the androgen receptor gene prevent the normal development of both internal and external male structures in 46XY individuals.

Typically, binding is absent in complete androgen insensitivity syndrome and binding affinity (Kd) is altered in partial and mild androgen insensitivity syndrome. Genital skin fibroblasts can be used to identify genomic mutations that disrupt normal RNA splicing, quantify androgen receptor expression by western blots, and study the phenotypic variance recorded between patients with the same mutation.[8,9] The gene responsible for the AIS phenotype is located on the proximal long arm of X Chromosome Xq 11-12. 70 % of mutations are X Linked recessive.

Clinical Features

CAIS presentation in newborn period is unremarkable and is absolutely feminised and a diagnosis at birth is not possible unless there is a discordance between prenatal sex determination by chorionic villi sampling or amniocentesis or maternal circulating free fetal DNA showing XY and postnatal appearance of normal female anatomy.[10] In infancy, complete androgen insensitivity syndrome presents as an inguinal hernia or labial swelling containing a testis in

an apparently female infant, karyotyping or a biopsy of a gonad within the hernial sac is done after the parents give consent. (Fig. 1)

Fig. 1 - Right inguinal hernia with gonad in a fully feminised child . Gonadal biopsy was done and the gonad placed in the abdomen.Biopsy was testis and lab investigations confirmed it as CAIS.

More often the diagnosis of CAIS is picked up when investigating an older girl with primary amenorrhoea. (Fig. 2)

Fig. 2 - A 14-year-old girl with CAIS presented with primary amenorrhoea. Testis in the inguinal region with normal vagina. Testis removed. At 24 years she got married and has normal sexual function

They have normal breast development; they are tall and hairless females with feminine external genitalia with short and shallow vagina. The gonads may be palpable in the inguinal canals. The vagina varies from a dimple in the perineum to normal length but is always blind-ending. The uterus, cervix, and proximal vagina are absent in complete androgen insensitivity syndrome because of the action of antimüllerian hormone produced by Sertoli cells of the testis.

Mild androgen insufficiency syndrome can also manifest as bulbar and spinal muscular atrophy (Kennedy's disease). In addition to the characteristic weakness and wasting of bulbar, facial and limb muscles, patients with this neurological disorder have increased testosterone concentrations and are associated with gynecomastia and reduced fertility, which is consistent with mild androgen insensitivity. The pathogenesis of bulbar and spinal muscular atrophy is associated with hyper expansion of the CAG repeat (more than 38) in exon 1 of the androgen receptor gene, leading to a toxic gain of function.[11]

Management

CAIS children are fully feminised at birth and reared as girls. Management of CAIS is currently limited to symptomatic treatment. Currently, there are no known methods to correct the Androgen receptor deficiency by AR gene receptors. The children are feminised at birth and do not need active treatment in the neonatal period. Parents of children with CAIS need considerable support in planning and implementing disclosure for their child once the diagnosis has been established and need proper counselling.

If the child is seen with inguinal hernia, diagnosis is arrived at by gonadal biopsy and other lab investigations to establish CAIS. (Fig 2)

If the gonads are left behind, they help in somatic growth and breast development due to aromatisation of testosterone into estrogen. As they near puberty the gonads can be removed by laparoscopy. When complete androgen insensitivity presents in infancy, early gonadectomy with puberty induction later can be done, or gonadectomy can be delayed until early adulthood. Parents might choose early gonadectomy at a time when their child is unaware of the issues surrounding a diagnosis of complete androgen insensitivity syndrome. The chance of tumour development before the age of 14 years is less and thereafter it increases. Prepubertal malignancy in

complete AIS is extremely rare. The cancer risk associated with CAIS is low enough to recommend against gonadectomy in the prepubertal period, although it is warned that the cancer risk is still elevated above the general population and that ongoing cancer monitoring is essential in children in whom the gonads are left behind. However, gonadectomy is necessary in post pubertal period since the chance of malignancy increases with increasing age. Studies have suggested an increased tumour risk of greater than 30% in late adulthood if gonadectomy is not done .[12]

If the gonads are removed early due to parental pressures or fear of malignancy, the child will need estrogen supplement for breast development and somatic growth. Estrogen replacement therapy is critical to minimize bone mineral density deficiencies later in life. Progesterone supplements are not necessary since the uterus is not present.

When the diagnosis is made at the time of puberty during investigations for primary amenorrhea, the management essentially boils down to the removal of gonads and replacement hormone therapy of estrogen. Most of the time the vagina that is present is adequate for normal sexual activity. If the length is less, then non-surgical self-dilatation using vaginal moulds is enough. If it is only a dimple, then they may need vaginoplasty preferably using bowel.

Psychosocial Aspect of CAIS

Most individuals with CAIS are raised as females. They are born phenotypically female and usually have a heterosexual female gender identity. The ideal time for disclosure of the genetic type of sex to the child is approximately 15 years. Disappointment and frustration can happen when they realise that they cannot bear children. Initial disclosure of the problem must be in detail to the parents and the parents with the physician may decide the appropriate way and time to

disclose to the child.

By and large, they have normal sexual activity and gender dysphoria is uncommon though some have dyspareunia due to the introitus being narrow.

Partial Androgen Insensitivity Syndrome (PAIS)

Aetiology is similar to CAIS where the response to androgen receptors is partial and is also an X linked recessive condition. The partial unresponsiveness of the cell to the presence of androgenic hormones impairs the masculinisation of the developing foetus, as well as the development of male secondary sexual characteristics at puberty. A number of cases of XY DSD are loosely labelled as 'PAIS' when no conclusive biochemical or genetic abnormalities are identified in gonadal function, androgen synthesis or androgen action. The term PAIS should be reserved for those children who have XY DSD and a pathogenic mutation in AR.

Fig. 3 - PAIS with clitoris like diminutive penis with hypospadiac urethra and empty labioscrotal folds

Differential Diagnosis

The children with PAIS have a micropenis, unfused labioscrotal folds, penoscrotal hypospadias and rudimentary vagina. The clinical picture may be that of minimal enlargement of phallus and genitalia that are predominantly like that of a male child. (Fig. 3)

The external appearance may simulate 5 Alpha reductase deficiency, 17 hydroxysteroid dehydrogenase deficiency and Leydig cell Hypoplasia. Measurement of Testosterone and Dihydrotestosterone before and after HCG injection may help to

differentiate individual conditions.

Older children reared as girls in PAIS may present with primary amenorrhoea, breast development with enlarged phallus and inguinal masses. Mullerian structures are not present in them. (Fig 4.)

Fig. 4 - PAIS reared as a girl showing virilization of external genitalia with normal breast development and underwent gonadectomy, phallus reduction and reconstruction

Quigley graded the severity of defective masculinisation.[13] He described the scale as one depicting "severity" or "defective masculinization". Grade 1 is indicated when the external genitalia is fully masculinized and corresponds to mild androgen insensitivity syndrome. Grades 6 and 7 are indicated when the external genitalia is fully feminized, corresponding to complete androgen insensitivity syndrome. Grades 2 through 5 of the Quigley scale quantify four degrees of increasingly feminized genitalia that correspond to partial androgen insensitivity syndrome. Grade 7 is indistinguishable from grade 6 until puberty and is thereafter differentiated by the presence of secondary terminal hair. (Fig 5)

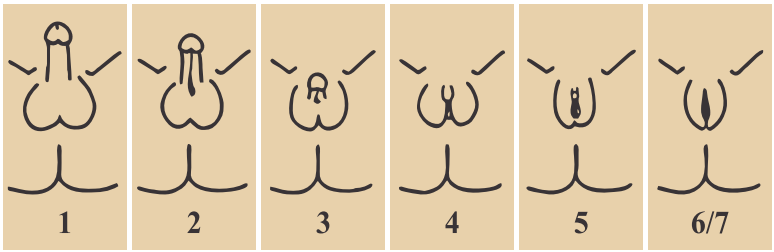


Fig. 5 - The first six grades of the scale, grades 1 through 6, are differentiated by the degree of genital masculinization. (Quigley C)[13]

Sex Assignment

Whereas in CAIS the children are fully feminised, in PAIS there is some virilization of the external genitalia. The decision of whether to raise an individual with PAIS as a boy or a girl may not be obvious at birth. If reared as a boy it is difficult to predict whether the penis would grow in view of the defective androgen receptors. If reared as a girl the cerebral imprinting of the brain in utero by the androgens may make the child have gender dysphoria and may have a considerable degree of dissatisfaction in sexual life.

Younger the child with minimal androgenisation of the external genitalia, it is preferable to rear them as girl children. An infant with partial androgen insensitivity syndrome who is assigned a female will need a genitoplasty and gonadectomy before puberty to avoid the risk of virilization. Oestrogen replacement is needed to induce feminisation at puberty.

Psychological distress is more common in adults with partial androgen insensitivity syndrome than in those with complete androgen insensitivity syndrome, irrespective of whether they were raised male or female.

Most of the PAIS children are reared as boys. Due to androgen receptor deficiency, the virilization potential during puberty is unpredictable. Virilization potential can be assessed by exogenous

administration of testosterone and dihydrotestosterone , while others have measured the change in sex hormone binding globulin (SHBG) in response to the artificial androgen stanozolol to assess androgen sensitivity.[14] Some experts have cautioned that it remains to be proved that a good response to exogenous androgens in neonates is a good predictor of androgen response at puberty. If a mutation in the AR gene is found, it is important to determine whether the mutation is inherited or de novo (i.e. a somatic mutation); a certain amount of the wild-type androgen receptor will be present in cases of somatic mutation, which can induce virilization at puberty. A genital skin fibroblast study and a human chorionic gonadotropin (hCG) stimulation test may also provide information.

While decision making in rearing sex in CAIS is not a problem, PAIS needs a lot of explanation to the parents of an affected newborn and, finally, to develop a management plan that leads to optimal long-term outcomes. An unequivocal diagnosis confirmed by biochemical and genetic means is necessary to counsel the parents regarding rearing sex in PAIS.

Key Points

- Androgen insensitivity syndrome (AIS) is characterised by the female phenotype in an XY individual due to androgen resistance as a result of mutations in the X linked androgen receptor.
- Complete androgen insensitivity syndrome (CAIS) is a condition where the phenotype of the child or older infants are absolutely feminine. When it is partially virilized it is called Partial androgen insensitivity syndrome (PAIS) and when it is mild form in boys or adults with gynaecomastia or infertility it is called Mild androgen insensitivity syndrome.
- All CAIS should be reared as girls.

- The gonads in CAIS children can be left alone till puberty for normal feminisation and thereafter it should be removed to prevent them from becoming malignant.

- When the diagnosis is made at the time of puberty, during investigations for primary amenorrhea, the management essentially boils down to the removal of gonads and replacement hormone therapy of estrogens.

- In PAIS the clinical picture may be that of minimal enlargement of phallus and genitalia that are predominantly like a male child and most of them are reared as male children.

- A number of other 46 XY disorders have similar clinical pictures like PAIS and genetic tests to see the mutation of the androgen receptor gene are final in PAIS.

- Both CAIS and PAIS need social and psychological support and parental guidance.

References

1) Wilkins L Heterosexual development in the diagnosis and treatment of endocrine disorders in childhood and adolescence Springfield IL Charles C Thomas 1950 Page 256 -279.

2) Lyon MF, Hawkes SG (September 1970). "X-linked gene for testicular feminization in the mouse". Nature. 227 (5264): 1217–9.

3) Migeon BR, Brown TR, Axelman J, Migeon CJ (October 1981). "Studies of the locus for androgen receptor: localization on the human X chromosome and evidence for homology with the Tfm locus in the mouse". Proceedings of the National Academy of Sciences of the United States of America. 78 (10): 6339–43.

4) Brown TR, Lubahn DB, Wilson EM, Joseph DR, French FS, Migeon CJ (November 1988). "Deletion of the steroid-binding domain of the human

androgen receptor gene in one family with complete androgen insensitivity syndrome: evidence for further genetic heterogeneity in this syndrome". Proceedings of the National Academy of Sciences of the United States of America. 85 (21): 8151.

5) Lubahn DB, Brown TR, Simental JA, Higgs HN, Migeon CJ, Wilson EM, French FS (December 1989). "Sequence of the intron/exon junctions of the coding region of the human androgen receptor gene and identification of a point mutation in a family with complete androgen insensitivity". Proceedings of the National Academy of Sciences of the United States of America. 86 (23): 9534.

6) Morris JM The syndrome of testicular feminization in male pseudohermaphrodites. Am J Obstet Gynecol. 1953; 65: 1192-1211.

7) Prof leuan A Hughes MD Androgen insensitivity syndrome SEMINAR Volume 380 issue 9851 p1419-1428 October 20 2012.

8) Wang M Wang J Zhang Z et al.Dissecting phenotypic variation among AIS patients.Biochem Biophys Res Commun. 2005; 335: 335-342.

9) Holterhus PM Deppe U Werner R Intrinsic androgen-dependent gene expression patterns revealed by comparison of genital fibroblasts from normal males and individuals with complete and partial androgen insensitivity syndrome.

10) Chiu RW Lo YM Lo D Non-invasive prenatal diagnosis by fetal nucleic acid analysis in maternal plasma: the coming of age. Semin Fetal Neonatal Med. 2011; 16: 88-9.

11) Finsterer J Bulbar and spinal muscular atrophy (Kennedy's disease): a review. Eur J Neurol. 2009; 16: 556-561.

12) Deans R Creighton SM Liao LM Conway GS Timing of gonadectomy in adult women with complete androgen insensitivity syndrome: patient preferences and clinical evidence. Clin Endocrinol. 2012.

13) Quigley CA, De Bellis A, Marschke KB, el-Awady MK, Wilson EM, French FS (June 1995). "Androgen receptor defects: historical, clinical, and

molecular perspectives". Endocr. Rev. 16 (3): 271–321.

14) Holterhus PM Sinnecker GH Hiort O Phenotypic diversity and testosterone-induced normalization of mutant L712F androgen receptor function in a kindred with androgen insensitivity. J Clin Endocrinol Metab. 2000; 85: 3245-3250.

Chapter 6

Mixed Gonadal Dysgenesis

Mixed gonadal dysgenesis (MGD) is a rare intersexual disorder, characterized in most cases by the presence of testis and a contralateral streak gonad; in some cases, the contralateral gonad may be rudimentary not having differentiated into an ovary or into a testis and in other cases, it may be absent.

Arthur Sohval described the entity and coined the term Mixed Gonadal dysgenesis based on his observation in five of his patients. He described that karyotype showed XO component in each case with a "streak" gonad on one side and an intra-abdominal testis on the other.[1]

The overall condition of the gonads in these cases apparently represents an intermediate form between "pure" gonadal dysgenesis on one hand and true hermaphroditism on the other. Situated intra abdominally in the normal position of ovaries, these consist of a rudimentary or vestigial "streak" gonad on one side and a testis on the other. Since the former is identifiable as neither testis nor ovary, such cases cannot be properly categorized based on gonadal histology as male pseudohermaphrodites .[1]

MGD is a syndrome characterized in most patients by a mosaic 45X/46XY or a 46XY karyotype, an abnormal testis, and a contralateral streak gonad. There is incomplete differentiation of testis resulting in incomplete inhibition of Mullerian development,

incomplete support of differentiation of the mesonephric duct structures, incomplete virilization of the external genitalia, and failure of the descent of the testis. There is a mixture of masculine and feminine features in an individual in whom neither gonad is normal. The pathogenesis of MGD is due to some inadequacy of a Y chromosome-related inductive factor in the testis.

The external genitalia is always masculinized to some extent, on occasion achieving a normal male phenotype and somatic signs of Turner's syndrome are frequently present. These patients are always chromatin-negative, and appear to represent the commonest expression of the mosaicism of XO and XY cells, probably resulting from a cytogenetic error very early in embryogenesis and they have persistent Mullerian structures. Testicular androgenic function appears to be quantitatively and qualitatively normal at puberty in MGD, despite complete lack of germ-cell proliferation. The discrepancy between normal Leydig cell function at puberty and the evidence of incomplete genital masculinization during fetal life may be explained by delayed and asynchronous fetal testicular development.[2]

Mixed gonadal dysgenesis and dysgenetic male pseudohermaphroditism (DMP) share similar features. It is suggested that these two disorders represent different spectra of the same disorder. A unifying concept of etiopathogenesis is proposed. Gonadal asymmetry in MGD is cytogenetically due to local prevalence of cell lines carrying different karyotypes; XO in the streak gonad and XY in the dysgenetic testis while sex chromosome abnormalities in the blood do not always reflect the genital abnormalities. The DMP has XY cell line and both the gonads are testis but are streak gonads. [3]

Pathophysiology of the Gonads in MGD

In a study of 21 cases of MGD by Rabboy et al[4], the following pathological aspects of the streak and other gonad were found. The gonads in 15 patients consisted of a macroscopic testis and a streak gonad; six patients had variants, including two with bilateral testes and four with bilateral streak gonads or tumours.

Testicular Pathology in MGD

Although gonads with seminiferous tubules usually developed to a moderately advanced state, macroscopically resembling testes, the hilar zone remained architecturally disorganized; the cortex invariably lacked more than a rudimentary tunica albuginea or exhibited partial ovarian differentiation, sometimes even with a rare primordial follicle. Over time, the seminiferous tubules atrophied and hyalinized. Gonads that grossly resembled streak gonads were observed microscopically to be composed of a stroma resembling that of the normal ovarian cortex. In patients more than several years of age, the entire complement of germ cells in streak gonads disappeared.[4]

Testes show the following features 1) Failed to completely inhibit müllerian development 2) Failed to support full differentiation of mesonephric duct structures 3) Failed to adequately masculinize development of the external genitalia or 4) Often failed to mediate their own descent, resulting in asymmetry of the internal and external genitalia.[4]

Pathology of Streak Gonads

None of the streak gonads mediated normal female adolescent development or fertility. Microscopic examination revealed that every gonad regardless of its gross appearance was morphologically abnormal.

A striking and consistent finding in the MGD syndrome is persistent Mullerian duct derivatives are present in 95 percent of patients.[5] Nearly every streak gonad (95 percent) was associated with an ipsilateral fallopian tube. Even gonads that grossly resembled testes were frequently associated with a fallopian tube (74 percent), suggesting that the testes are functionally and morphologically abnormal, secreting little or no Mullerian inhibiting substance or secreting it too late to affect the developing tube.[5]

Fertility

The uterus is found on the side of the streak gonad. More often it is abnormally attenuated and without the lumen.[4] The testis does not produce sperm and the streak ovaries become fibrotic over time. Fertility is not possible. Moreover, the gonads are removed early in life to prevent them from undergoing malignancy. Hence fertility is not possible whether the child is reared as boy or girl.[6]

If the patient is to be raised as a female, early removal of the gonad also prevents the masculinisation at puberty due to the secretion of androgens by the testis.

MGD and the risk of malignancy in the gonads

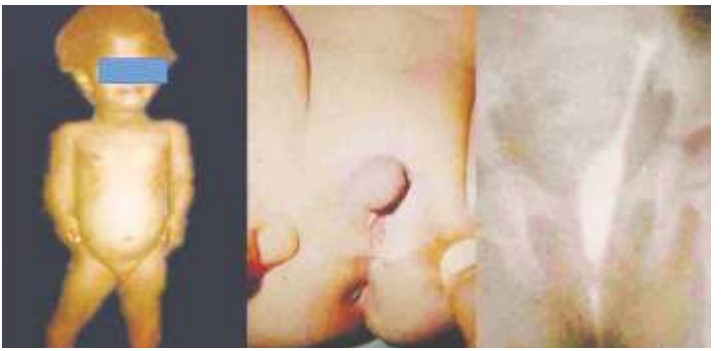
Neoplastic transformation, so characteristic of this group of patients, may result from unprotected germ cells and abnormally high and prolonged gonadotropin stimulation. Gonadoblastoma and seminoma-dysgerminomas are the tumours found in the gonads with the risk exceeding 50% as the third decade is approached.[6] The neoplastic changes occur with Y cell line and the predisposition is due to high levels of circulating gonadotrophic hormones FSH and LH. Risk of tumour increases with age and 20 % by 15 years and 75 % when they reach 26 years.[7]

In DSD children with 46XY karyotype, carcinoma in situ (CIS) occurs in dysgenetic testes in all cases and is frequently associated with gonadoblastoma. Impaired organogenesis of sex cords, relative inhibition of testosterone secretion, and the associated increased secretion of gonadotropins may create a milieu that induces or is favourable for the formation or maintenance of neoplastic lesions in dysgenetic testes early in childhood.[8] Since a gonadoblastoma is often microscopic, it is not always detected clinically or by palpation of the gonad. For these reasons excision of both gonads at an early age is considered mandatory.

Clinical Features

The external appearance of the genitalia is ambiguous. They may be normal appearing female children with clitoromegaly and absent gonads in the labia. XO configuration induces short stature and other features of Turner's syndrome. Labio scrotal folds are open and vaginal orifice is seen. Ultrasound abdomen will show the rudimentary uterus and the streak gonad on one side and normal to dysgenetic testis on the other side (Case 1).

Case 1.



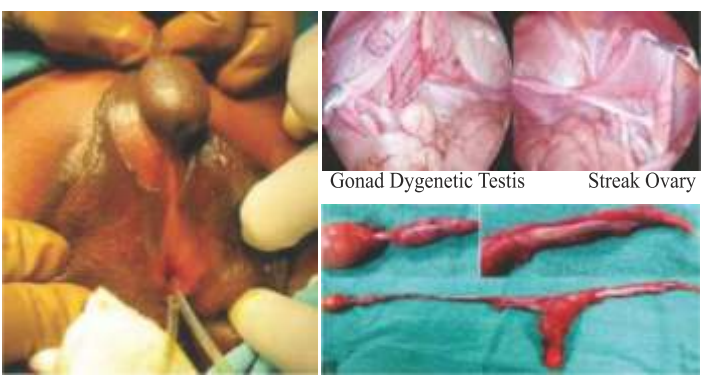
Case 1 - MGD reared as a girl with short stature, enlarged phallus with vagina and rudimentary uterus

A two year old MGD child reared as a girl with enlarged phallus with vagina was seen. The genitogram showed roomy vagina and cord like uterus and diminutive fallopian tube. Short stature and webbing of neck characteristic of XO configuration was seen. The child was treated by external genitalia feminisation and the gonads were removed. The uterine remnant was left behind.

Phenotype males will have enlarged phallus, hypospadiac urethra short stature, bilateral undescended testis. Ultrasound may show the streak gonad and the testis and the Mullerian remnants.

Case 2.

14-year-old boy was seen with hypospadias with severe chordee, with bilateral absence of gonads. The scrotal folds were unfused. The vaginal and urethral orifice were seen separately. Laparoscopy showed streak gonad on one side and testis on the other side' The testis was without epididymis and vas. The rudimentary uterus was also seen. Bilateral Gonadectomy and removal of uterine remnants and hypospadias repair was done. He will receive testosterone supplements and will have insertion of testicular prosthesis at the time of puberty.



Case 2 - MGD reared as boy showing hypospadiac urethra with rudimentary vagina and empty scrotum. Laparoscopic view of Left testis without epididymis and right streak gonad with rudimentary uterus is seen. The uterine remnant with the gonads were removed

Differential Diagnosis

MGD must be differentiated from anomalies such as Ovo testicular DSD (true hermaphroditism,) testicular regression syndrome and androgen insensitivity syndrome, in which gender change may not be necessary and gonadal removal is not imperative, at least while the patient is very young. The differentiation of Ovo testicular DSD from MGD is important. A bilateral gonadectomy is recommended in all individuals with MGD containing Y-chromosome material. In Ovo testicular DSD, however, only the removal of the opposite gonad from the assigned gender and a biopsy of remaining gonadal tissue for histological evaluation may be appropriate. Secondly, gender assignment is critical for the treatment of patients of Ovo testicular DSD because these patients usually do not possess any other developmental malformations, and thus normal sexual and reproductive functions can be achieved by proper management and sex assignment at a young age .[5]

In MGD, the ovarian component, if present, is microscopic and does not develop beyond primordial follicles. The ovarian gonad in ovo-testicular DSD macroscopically resembles normal ovary and is frequently functional, as has been evidenced by menstruation and reports of pregnancy.[9]

The other differential diagnosis is Dysgenetic male pseudohermaphroditism which is considered as a variant of MGD. The gonads that are streak like are testis bilaterally and the cell line is XY and they have Mullerian structures. Clinically the scrotum is empty with partial labioscrotal fusion with diminutive phallus. The gonads have a high propensity to become malignant. [10]

Most of them are reared as girls and need removal of gonads and rudimentary uterus.

Gender Assignment and Management

Gender assignment in MGD is based on the potential for normal sexual function and decided by the parent and the team looking after the child. Laparoscopy aids both in diagnosis as well as definitive surgical treatment. Close follow-up is mandatory for detecting the highly prevalent gonadal tumour in those children reared as boys if the gonads are left behind.

Children with MGD born feminised but with slight enlargement phallus need genitoplasty and removal of the gonads by laparoscopy since the chance of gonadoblastoma is high. Leaving behind the testicular component may produce virilization during puberty. Regarding the Mullerian derivatives, the infantile uterus has no lumen in most of the cases. The question more often arises is “should the uterus be retained and primed with estrogen to become a surrogate uterus to receive the fertilised ovum in later life?” One of Robboy's case, patient received estrogen therapy for nearly two decades after gonadectomy and developed an adenosquamous carcinoma of the endometrium. Although rare, this complication has been reported in women with Turner's syndrome who are on long-term estrogen therapy. If the uterus is retained to lower the risk of carcinoma or to detect it in an early or precursor state, it has been suggested that the estrogen replacement therapy should consist of estrogen-progesterone combinations.[11,12]

Older children reared as boys need correction of hypospadias, removal of streak gonad and also the intra-abdominal testis. If an orchiopexy is done or if the gonad is retained in the scrotum a biopsy of the gonad is necessary to see for evidence of carcinoma in situ. Constant vigil by self-examination and ultrasound guidance is necessary to exclude malignancy. When both gonads are removed in the initial surgery itself children will need androgen supplements and insertion of the testicular prosthesis at the appropriate age. Short

stature due to Turner like condition can be corrected by growth hormone replacement.

The management of Dysgenetic male Pseudohermaphroditism is similar to the management of MGD since it carries the same risk of malignancy. The uterus is usually hypoplastic and non-functional and not worthwhile conserving.

Key Points

- MGD is a syndrome characterized in most patients by a mosaic, 45XO, 46XY an abnormal testis, and a contralateral streak gonad.
- There is a mixture of masculine and feminine features in an individual in whom neither gonad is normal. The pathogenesis of MGD is due to some inadequacy of a Y chromosome-related inductive factor in the testis.
- The testes are functionally and morphologically are abnormal. The streak gonad will have fibrous stroma with few ovarian follicles. A rudimentary uterus is present on the side of streak gonad.
- Clinically the child may present with slight phallus enlargement bilateral empty scrotal folds with unfused labio scrotal folds with urethra and vagina seen separately. In masculinised children, they may resemble a child with penoscrotal hypospadias with bilateral undescended testis.
- The gonads have a high propensity to become malignant and the incidence of gonadoblastoma increases with increasing age.
- Children reared as girls will need removal of both gonads and feminisation of external genitalia. The uterine remnant if left behind and if the individual is on estrogen one has to watch for the development of malignancy. Most of the time the uterus is infantile

without a lumen and leaving it to become a surrogate uterus in later life is questionable.

- If reared as boys, they need hypospadias repair and removal of intra-abdominal gonads and replacement of hormones and application of testicular prosthesis. If the testis is in the scrotum or orchiopexy was done constant vigil is necessary to exclude malignancy in the retained testis.

References

1) Arthur R. Sohval"Mixed" Gonadal Dysgenesis: A Variety of Hermaphroditism. Am J Hum Genet. 1963 Jun; 15(2): 155–158.

2) Frank Davidoff, Daniel D. Federman, Mixed gonadal dysgenesis Pediatrics November 1973, volume 52 / issue 5 .

3) Rohatgi M1, Gupta DK, Menon PS, Verma IC, Mathur M. Mixed gonadal dysgenesis and dysgenetic male pseudohermaphroditism--a critical analysis. Indian J Pediatr. 1992 Jul-Aug;59(4):487-500.

4) Stanley J. Robboy, MD, Theodore Miller, MD, Patricia K. Donahoe, Dysgenesis of testicular and streak gonads in the syndrome of mixed gonadal dysgenesis: Perspective Derived from a Clinicopathologic Analysis of Twenty-one Cases Hum Pathol 13:700- 716, 1982.

5) Donahoe PK, Crawford JO, Hendren WH: Donahoe PK, Crawford AD, Hendren Mixed gonadal dysgenesis: pathogenesis and management. J Pediatr Surg 14:287, 1979.

6) Zach W, Kalderon AE, TucciJR: Mixed gonadal dysgenesis: a case report and review of the world literature. Acta Endocrinol [Sup pI] 197:3, 1975.

7) Manuel M, Katayama PK, Jones HW Jr The age of occurrence of gonadal tumors in intersex patients with a Y chromosome. Am J Obstet Gynecol. 1976 Feb 1;124(3):293-300.

8) Słowikowska-Hilczer J1, Szarras-Czapnik M, Kula K Testicular

pathology in 46, XY dysgenetic male pseudohermaphroditism: an approach to pathogenesis of testis cancer. J Androl. 2001 Sep-Oct;22(5):781-92.

9) Morishima A, Grumbach MM: The inter-relationship of sex chromosome constitution and phenotype in the syndrome of gonadal dysgenesis and its variants. Ann NY Acad Sci155:695, 1968.

10) Rajfer J, Mendelsohn G, Arnheim J, Jeffs RD, Walsh PC. Dysgenetic male pseudohermaphroditism. J Urol. 1978 Apr;119(4):525-7.

11) Rosenwaks Z, Wentz AC, Jones GS, et al: Endometrial pathology and estrogens. Obstet Gynecol 53:403, 1979.

12) Benjamin I, Block RE: Endometrial response to estrogen and progesterone therapy in patients with gonadal dysgenesis. Obstet GynecoI50:136, 1977.

Chapter 7

Ovotesticular DSD

Ovotesticular DSD (OT-DSD) known as true hermaphroditism, is a rare condition in which an individual is born with ovarian and testicular tissue. More commonly one or both gonads is an ovotestis containing both types of tissue.

In Greek mythology, Hermaphroditus or Hermaphroditos - Ancient Greek : was the son of Aphrodite and Hermes. According to Ovid, he was born a remarkably handsome boy with whom the water nymph Salmacis fell in love and prayed to be united forever. The God, in answer to her prayer, merged their two forms into one and transformed them into an androgynous form was portrayed in Greco-Roman art as a female figure with male genitals. The OT-DSD is a rare disorder that has worldwide occurrence but is more common in black races especially in Africa.[1]

The Cause

- There are several ways in which this may occur and many theories have been proposed.
- It can be caused by the division of one ovum, followed by fertilization of each haploid ovum and fusion of the two zygotes early in development.
- Alternately, an ovum can be fertilized by two sperm followed by trisomic rescue in one or more daughter cells.
- Two ova fertilized by two sperm cells will occasionally fuse to

form a tetra-gametic chimera. If one male zygote and one female zygote fuse, a hermaphroditic individual may result. This may explain 46 XX/ 46 XY Karyotype.

- It can be associated with a mutation in the SRY gene.[2]
- Translocation of sequences such as Sex determining gene Y (SRY) on to the X chromosome resulting in differentiation of testis in 46XX DSD
- Development of testis in the absence of the SRY gene by a mutation in the X chromosome and also by occult mosaicism.

Karyotype in Ovotesticular DSD

Primary karyotypes for OT-DSD are XX with genetic anomalies (55-70% of cases), XX/XY (20-30% of cases) and XY (5-15% of cases) with the remainder being a variety of other chromosomal anomalies and mosaicisms.

Types of Ovotesticular DSD

OT-DSD occurs when an ovary and a testis or a gonad with mixed histologic features (ovotestis) is present.

Four categories are recognized by von Niekerk and Retief.[2]

- Bilateral, with testicular and ovarian tissue (ovotestis) anatomically present on each side; 20.8%
- Unilateral, with an ovotestis on one side and a normal ovary (29.1%) or testis (11%) on the contralateral side
- Lateral, with a testis is evident on one side and an ovary on the opposite side; 29.6 %
- Indeterminate, in which the clinical syndrome is expressed but the location and type of gonadal tissue is uncertain. 9.5 %[2]

The Ovo testis is more common on the right-side. Ovary is more common on the left and the testis on the right side.[2]

Pathogenesis of the Gonads

The anatomical site of the gonads is predominantly determined by the histology of the gonad whether it is ovary or testis. Gonads with a high percentage of testis material are typically found in the scrotal sac, while gonads with more ovarian tissue can be found in the abdomen.

A majority (47 %) of the ovotestis is in the abdomen and 24 % are inguinal and 26 % were in the labia.[2] If the ovarian component is more than the testicular component the gonad is intra-abdominal and if the testicular component is more it is more commonly seen in inguinal or labioscrotal fold.[2,3]

It is extremely rare to find the ducts of both Wolffian and Müllerian origin next to an ovotestis and such occurrence has not been reported.[3]

Both the size and the amount of testicular tissue determine the virilization of the internal and external genitalia. The development of a uterus and fallopian tubes can occur in the presence of an ovary or ovotestis. However, the male Wolffian structures require a well-formed testis to fully develop.

The bisexual gonad contains testicular tissue with distinct tubules, and the ovarian tissue has follicles. However, the ovarian tissue must contain oocytes for the diagnosis; the presence of only ovarian stroma is not an adequate criterion. With increasing age, ovarian tissue is often normally developed and pregnancies have been reported. The testicular portion is often immature and spermatogenesis is rare.

In the ovotestis, the testicular tissue may be hilar or more often they lie end to end. The ovarian tissue is firm and slightly irregular whereas the testis surface is smooth and glistening and soft to touch. While the ovarian tissue is normal histologically showing evidence of ovulation, the testis shows immature tissue with a lack of spermatogonia and spermatogenesis. Older children may show progressive sclerosis of testicular tissue and reduction in function.

The Risk of Malignancy

The risk of malignancy in ovo-testicular DSD ranges from 2.6% to 4.6% and it is lower than in other types of DSD.[2] Since the chance of malignancy is low, prophylactic removal of the gonad is not indicated.[1] The most common neoplasm is a Germ cell tumour, dysgerminoma being the most common histological type.[2] In a case report, three gonadal neoplasms namely, yolk sac carcinoma, gonadoblastoma and seminoma has been reported in the same patient, while the ovary on one side was replaced by yolk sac carcinoma, the ovotestis was partly destroyed by the other two. The cytogenetic study revealed a hypodiploid number and a mosaic sex chromosomal pattern.[4] Breast carcinoma in older individuals with XX true hermaphroditism has been reported.[5]

Clinical Features

The main clinical features depend on the time of presentation. In infancy when they present early, they have varying degrees of hypospadias and cryptorchidism is common, but at least one gonad is palpable, usually in the labioscrotal fold or inguinal region. They are often associated with an inguinal hernia. Ultrasound examination will show the other gonad as well as the uterus if present.

If the child has been reared as a boy, at puberty the ovarian component may induce gynecomastia, and cyclical menstruation presenting as cyclical haematuria. This is due to excessive oestrogen secretion by the ovary or ovotestis. If reared as girls at the time of puberty the testicular component may induce virilization of the external genitalia and voice changes.

Diagnosis

Ultrasound examination of the abdomen should be the first line of investigation of the non-palpable testis. If the ultrasound imaging is

inconclusive, MRI examination should be done which offers excellent soft tissue contrast without exposure to radiation. However, both testes and non cystic immature ovaries have similar signal intensity on T1- and T2-weighted images. Ultrasonic textural differences between testes and ovaries are well recognized. Undescended gonads may pose a challenge to the ultra-sonographer because of the small size and echographic pattern that is similar to the adjacent tissues.[4] MR imaging helps to characterize the abnormal pelvic anatomy.

Patients with XX testicular DSD have hypergonadotropic hypogonadism with elevated follicle-stimulating hormone (FSH) and LH, decreased T and DHT, and a less than twofold increase in response to the hCG-stimulation test. Unlike individuals with XX testicular DSD, the gonads of patients with ovotesticular DSD have some degree of function and therefore have normal levels of FSH, LH, estradiol (E2), T, and DHT.

Genetic tests that should be performed are FISH for SRY, which is positive in 90% of phenotypically male patients with XX testicular DSD. Chromosomal microarrays will also detect the presence of SRY and should therefore be prioritized, especially in cases of genital ambiguity. In cases of SRY translocation, the nature of the rearrangement should be evaluated (translocation to X vs. autosome). It is important to note that the only way to obtain a definitive diagnosis of either ovotesticular or testicular DSD is by gonadal tissue confirmation in which both gonads are extensively examined for the presence of ovarian tissue.

Laparoscopy is also helpful in diagnosing patients with ovo testicular DSD, the gonads and internal anatomy such as the uterus, fallopian tubes can be seen well. Biopsy of the gonad can be done during initial laparoscopy. Planning of corrective surgery such as removal of the gonad and uterus can be done after the biopsy report.

Management

The decision of sex of rearing in patients with ovotesticular DSDs must take into account the potential for normal sexual function and fertility based on the degree of gonadal differentiation and genital development.

Children Reared as Girls

The ovaries in Ovotesticular DSD are functional and if they are reared as girls, they can give birth to children. The testis on the other hand shows histological abnormality and undergoes slow degeneration. Though occasional pregnancies have been reported they are usually not fertile. In infancy, the child can be reared as a girl if uterus and vagina is present with ovaries. In these female-reared patients, partial gonadectomy limited to the testicular component must be performed in infancy during reconstructive surgery. If there is ovary on one sided and testis on the other side removal of testis after histological confirmation is adequate. If it is ovary on one side and ovotestis on the other side removal of ovotestis is the choice. In bilateral ovotestis, the differentiation between the testis and ovary may be challenging. The testis appears smooth, glistening and yellow and is soft to touch. The ovary is convoluted, dull and firm to touch. Initial laparoscopic gonadal biopsy on either side with markers to identify the ovarian and testicular area is done. After the histological proof the testicular component is removed. Histologically it is advisable to cut on the ovarian side of the polar ovotestis to ensure complete removal of the testicular component. Its complete removal is confirmed by and monitored postoperatively by measuring serum AMH levels or by demonstrating a lack of testosterone response to HCG stimulation. Pregnancy is possible and normal infants have been delivered.

Children Reared as Boys

If the child is reared as a boy based on good phallic size and descended gonad on one side, the hemi-uterus and all ovarian tissue has to be

removed to prevent cyclical haematuria and gynecomastia. If the ovary and testis are separate it is easy to remove the unwanted gonad. But in ovotestis, ovarian component has to be removed and may be challenging. The external genitalia needs correction of chordee, urethral advancement and orchiopexy. Ovotesticular DSD males are generally azoospermic, but there have been infrequent cases of males with mature sperm and in rare instances, ovotesticular DSD when reared as men have fathered children.[6]

Though self fertilisation has been well documented in mammals with true hermaphrodites, Bayraktar Z analysed the possibility of potential auto fertility in true hermaphrodites in humans though it has not been reported .[7]

Case Report

A three-year-old male child was seen with right undescended testis. (Fig. 1) He had descended left testis and also had a urethral fistula in the mid penile region. The fistula was present from birth. An ultrasonogram of the abdomen showed that the right gonad was intra abdominal with uterus on the right side. A karyotype was 46 XX. Laparoscopy showed a well developed gonad and rudimentary uterus on the right side. (Fig 2.) The left side vas was seen entering the deep ring and right gonadal biopsy was done. Gonadal biopsy on the right

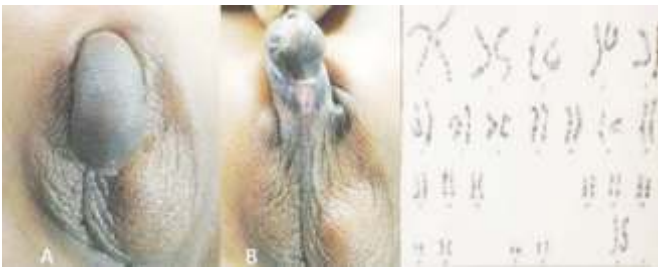


Fig.1 - Ovo testicular DSD reared as boy. Three-year-old child reared as a boy with good phallus, descended testis with midpenile congenital urethral fistula. Karyotype shows 46 XX.

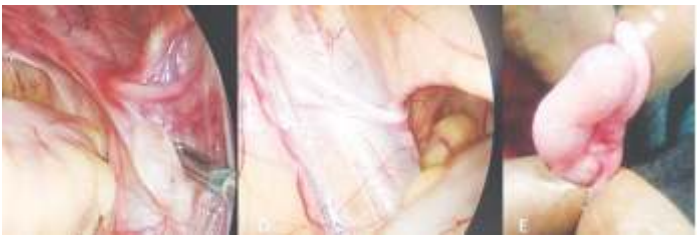


Fig. 2C. Laparoscopic view of the rudimentary uterus and gonad on the right side which turned out to be ovary on biopsy.
Fig. 2D. Laparoscopic view of the left testicular vessels and vas entering the deep ring.
Fig. 2E. The normal testis on the left side exposed and biopsied to exclude ovotestis

side showed that it was ovary. The parents were counselled the rearing sex was determined as a male child.

Repeat laparoscopy was done and the ovary and uterine remnant on the right side was removed. The urethral fistula was corrected. The left gonad was exposed on the left side of the scrotum. It had normal epididymis. Biopsy was done on polar areas to exclude an ovotestis and the report was testis.

This is a classic case of Ovo testicular DSD and the child had 46 XX as karyotype with gonads of both sexes with the uterus. Since the child was already reared as a boy and had well developed phallus with descended testis on the left side and the parental wish was also of rearing the child as a male child, surgical procedures were carried out to remove the right ovary and uterus. The gonad on the left scrotum was explored to exclude the possibility of ovotestis. The biopsy showed that it was testis. The unusual feature of this chid was the associated congenital urethral fistula in the mid penile region which was corrected.

Key Points

- The current terminology of a true hermaphrodite is Ovotesticular DSD which is a rare anomaly, the incidence being higher in African population

- Ovotesticular DSD is a condition in which an individual is born with ovarian and testicular tissue. More commonly one or both gonads is an ovotestis containing both types of tissue.
- While the ovarian tissue is normal histologically showing evidence of ovulation, the testis shows immature tissue with a lack of spermatogonia and spermatogenesis.
- In children reared as a girl, pregnancy is possible whereas those reared as boys are usually sterile.
- In those reared as girls, the testis and testicular component of ovotestis must be removed to prevent virilization at puberty.
- Similarly, in those reared as boys, the ovary and uterus should be removed to prevent gynecomastia and cyclical menstruation manifesting as haematuria during puberty.
- The incidence of malignancy in the retained gonad is low though it has been reported.

References

1) Ramsay M, Bernstein R, Zwane E, Page DC, Jenkins T.: differentiation. Am J Hum Genet. 1988 Jul;43(1):4-13.

2) van Niekerk WA, Retief AE. The gonads of human true hermaphrodites. Hum Genet. 1981;58(1):117-22 .

3) Krob G1, Braun A, Kuhnle U. True hermaphroditism: geographical distribution, clinical findings, chromosomes and gonadal histology. Eur J Pediatr. 1994 Jan;153(1):2-10.

4) S. Radhakrishnan, L.Sivaraman. P.S, Natarajan.True hermaphrodite with multiple gonadal neoplasms. Report of a case Cancer 42(6):2726 - 2732, December 1978.

5) John P, Decker MD Harvey J Breast carcinoma in a 46, XX true hermaphrodite - Cancer, April 1982.

6) Younis JS1, Radin O, Kerner H, Ben-Ami M Successful monozygotic twin pregnancy fathered by a male 46, XY true hermaphrodite. Reprod Biomed Online. 2011 Jan;22(1):80-2.

7) Bayraktar Z1. Potential auto fertility in true hermaphrodite J Matern Fetal Neonatal Med. 2018 Feb;31(4):542-547.

Chapter 8

Persistent Mullerian Duct Syndrome

Persistent Mullerian Duct Syndrome (PMDS) is a rare anomaly characterized by the presence of Mullerian duct derivatives (i.e. uterus, cervix, fallopian tubes and upper two thirds of the vagina) in a phenotypically and karyotypically male patient.

Embryologically the indeterminate gonad in the foetus is converted into testis by the testicular determining gene. The foetal testis has two types of cells. The Leydig cell which secretes testosterone and has a direct effect on the Wolffian ducts and promotes their differentiation into the epididymis, vas deferens, and seminal vesicles. Dihydrotestosterone induces male differentiation of external genitalia. Sertoli cells secrete Anti Mullerian hormone (AMH), which leads to regression of the Mullerian ducts. PMDS patients have Wolffian structures and due to deficiency of Anti Mullerian hormone develop persistent Mullerian duct syndrome.

PMD syndrome is caused by the deficiency of fetal anti-Müllerian hormone (AMH) effect due to mutations of the gene for AMH or the anti-Müllerian hormone receptor, but may also be as a result of insensitivity to AMH of the target organ in otherwise normally virilized 46XY males. Mutation in the AMH gene (PMDS Type 1) or AMHR2 gene (PMDS Type 2) are the primary causes of PMDS.[1] The Mullerian ducts mesenchyme has two types of receptors. Of this type II receptor is specific for AMH and is transmitted as autosomal

recessive pattern. The parents of the child may show the mutant genes but are free of the condition.

There are Two Types of PMD Syndrome

a) PMDS type I results from mutations of the gene for AMH on the tip of the short arm of chromosome 19 band p 13.3.

b) PMDS type II is the result of mutations in the gene AMHR II for the AMH receptor on chromosome 12 (13.q.12).[2]

Two anatomic variations of PMDS have been described - male and female forms. The male form is encountered in 80-90% of cases, characterized by unilateral cryptorchidism with a contralateral inguinal hernia which is of two types. Type-I, hernia uteri inguinalis comprises one side descended testis with herniation of the ipsilateral corner of the uterus, fallopian tube into the inguinal canal. Type-II, crossed testicular ectopia where both testes, entire uterus, both fallopian tubes herniate into one side of inguinal canal and opposite inguinal canal and scrotum being empty.

The second anatomic variant of PMDS, the female form, is seen in only 10-20% of cases and is characterized by bilateral cryptorchidism, with the testes fixed within the round ligaments in an 'ovarian position' with respect to the uterus.[3 4, 5] The gonads are fixed within the pelvis. The mobility of Mullerian structures is an important factor that determines the clinical presentation. If the uterus and fallopian tubes are mobile, they may descend into the inguinal canal during testicular descent. On the other hand, if the Mullerian structures are relatively immobile, testicular descent may be impeded. [6]

The anatomy of the male excretory ducts deserves special attention. Initially, the vas deferens is present in the mesosalpinx and then reaches the uterine wall, eventually penetrating it to open at the top of the vagina, the anatomical equivalent of the prostatic utricle.The spermatic vessels are usually very short and must be divided at the

surgery to allow placement of the testis in the scrotum. This leaves the vascularisation of the testis at the mercy of the deferential artery, in close proximity to the Mullerian derivatives and therefore easily damaged by attempts to remove them. In PMDS, the vas is often abnormal, narrow, blind, or even absent. Epididymal dissociation from the testis is common. Abnormalities of male excretory ducts are often reported in the literature.[7,8]

The Mullerian derivatives are represented either as well-developed uterus or a hypoplastic one. The uterus and fallopian tube may be present in the hernia sac. It may remain in the pelvis and may be missed. It opens at the verumontanum or it may be closed completely.[9]

Clinical Features

There no specific signs and symptoms for PMDS. Most of them are diagnosed while operating for inguinal hernia or undescended testis and is called "hernia uteri inguinalis" While operating on an inguinal hernia when the sac is opened the uterus prolapses with the cord structures. Traction may deliver the opposite testis.

In crossed testicular ectopia both the testes are on the same side with the uterus. The uterus is infantile and fallopian tubes are short and hypoplastic. It is suggested that these testes do not have gubernacular attachments and hence they are not guided to the appropriate scrotum.

Alternatively, PMD syndrome is diagnosed during laparoscopy. The presence of Mullerian derivatives may be seen while laparoscopy is done for intra abdominal testis. In adults, it may present with intra abdominal malignancy in the retained gonad. Adults in whom cryptorchidism or inguinal hernia have been neglected may come to medical attention because of haematuria due to hormonal imbalance in aging patients whose testes produce less androgens and an excessive amount of estrogens.[10]

Malignancy in PMDS

33% of PMDS patients 18 years and older experienced some form of unilateral or bilateral malignant testicular degeneration.[11] Seminomas are the most frequent, but choriocarcinomas, mixed germ cell tumors, embryonal cell carcinoma, gonadoblastomas and yolk sac tumors have been reported.

Conventionally the uterus in PMD syndrome is left behind since the vas runs very close and penetrates to enter the vagina which opens at the verumontanum. It is believed that uterine malignancy will not develop since there is no estrogenisation of the uterus. However, malignancy in the retained uterine segment has been reported.[12] Haematuria is often the presenting sign. Benign tumours like uterine leiomyoma can develop in the uterus.[13]

Fertility

In PMDS infertility is common. There are inherent defects of spermatogenesis at an early age in PMDS. Farag reported a proportion of 11% of fertile patients in Kuwait and neighbouring populations.[14] Most of these patients presented with either transverse testicular ectopia or hernia uteri inguinalis, i.e., at least one testis was in a normal scrotal position. Fertility is rare but possible in PMDS provided the testis is descended and the excretory ducts are intact. All fertile PMDS patients fathered children before their condition had been diagnosed.

Diagnosis and Investigations

Diagnosis is more often incidental. Mullerian structures can be picked by ultrasound in a hernia with undescended testis or in bilateral intraabdominal testis. Ultrasound, CT scan and MRI have all been used especially for screening the siblings of which CT is more dependable. The enzyme estimations are usually normal though the circulating AMH levels may be low. The karyotype is 46 XY. Since

Mullerian structures are present in Ovotesticular DSD and mixed gonadal dysgenesis the differentiation from PMDS is obvious since the external genitalia in PMD is normal compared to other conditions that show genital ambiguity.

Management

The management options depend upon the way PMDS presents in a child. If the uterus is found incidentally during laparoscopy for undescended testis the orchiopexy will need a staged procedure. The gonadal vessels are short and the vas will be running along the sidewall of the uterus and opening into the vaginal area. Initially the gonadal vessels are clipped and subsequently, the uterine segment is split in the midline and the mucosa is striped and orchiopexy is done.[9] If the testis cannot be brought down in spite of staged surgery and adequate mobilisation, orchiectomy to be done, since the chance of the retained gonad to become malignant is high. If there is any doubt regarding the histology of the gonad, a gonadal biopsy is done.

In PMDS associated with crossed testicular ectopia the uterine segment is divided in the midline up to the cervix, the mucosa is removed and orchiopexy of the testis is done through the septum in the scrotum.[9] Occasional malignancy in the retained uterine segment is prevented by removing the mucosa.

In adults with missed PMD syndrome, in whom intra-abdominal testis was not attended to, may present with a mass in the abdomen due to malignancy in the intra abdominal testis. They need removal of the abdominal testis and the tumour with the uterus and fallopian tubes. (Fig 2)

Case Report - 1

A two year old child had impalpable testis clinically on the left side. An ultrasound showed that there was crossed testicular ectopia in the right scrotum. Through an inguinal incision on the right side both the

testes were delivered. A well developed uterus and fallopian tubes with testis on either side were seen. The testes did not have epididymis. (Fig 1) The surface was irregular unlike the normal testis which will have a glistening surface. Biopsy of both gonads was done

Fig. 1 - A. Child with crossed testicular ectopia. Both testes are on the right side B. Uterus and testes C. Testis without epididymis

and the uterus and gonads were pushed inside the abdomen and wound closed. The biopsy report was testis on both sides. The child was reexplored and the uterus was split in the midline and the mucosa was cored out as has been reported by El Gohari.[9] Orchiopexy was done through the septum of the scrotum.

Case Report - 2

A thirty year old man with bilateral undescended testis was seen with the mass in the abdomen on the left side. Laparotomy revealed a large tumour on the left side with the uterus and vagina. The right testis was normal but intraabdominal. The uterus and the tumour with the right

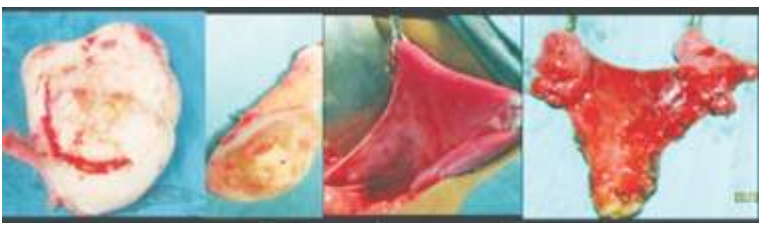


Fig. 2 - Testicular tumour on the left side with normal testis on the right side with the uterus

intra-abdominal testis and the left testis were removed. (Fig 2) Histologically the tumour was seminoma.

Case Report - 3

Case summary (Dr. Yogendra Sanghvi): 5yr/M presenting with bilateral non palpable testis with right inguinal hernia underwent laparoscopy. He had bilateral intrabdominal testes connected to a uterine structure with the vagina by a fallopian tube-like structure. The vas could be seen clearly running on either side of the uterine remnant. (Fig 3)

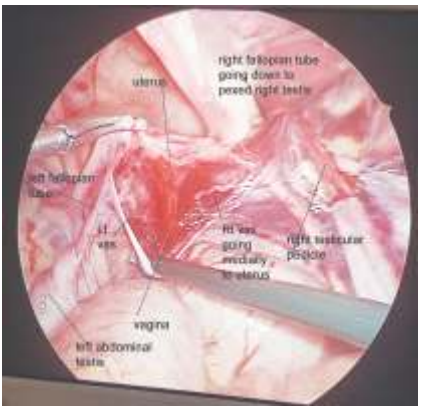


Fig. 3 - Laparoscopy showing Uterine remnant with vas on either side with bilateral intra-abdominal testis. (Dr.Yogendra Sanghvi)

Parents were consulted intraoperatively about the findings of female internal genital organs in the boy, which they did not take well in view of the child being otherwise normal boy with non-ambiguous genitals. Consent for the excision of the Mullerian system was not available even when they were told that that would result in bilateral successful orchiopexy.

With that constraint, after complete mobilization of the right testis, the right testis could come down well to do successful orchiopexy. However, this resulted in excessive traction on left testis preventing it from successful orchiopexy. Then on left side stage 1 Fowler Stephens was done. Right laparoscopic herniotomy was done by closing the right internal ring. Postoperative period was uneventful. Karyotype was 46XY. With that evidence now parents are willing to accept the diagnosis of PMDS. They are willing for excision of the Mullerian system at the time of Fowler Stephen's stage II after 6 months.

Key Points

- Persistent Mullerian Duct Syndrome is characterized by the persistence of Mullerian duct derivatives (i.e. uterus, cervix, fallopian tubes and upper two thirds of the vagina) in a phenotypically and karyotypically male patient. (XY)
- The external genitalia is well masculinised and thus differentiates it from Ovo testicular DSD and Mixed Gonadal dysgenesis who have Mullerian structures and show ambiguity in external genitalia.
- The diagnosis is incidental while surgery is being done for inguinal hernia or laparoscopy or laparotomy for intra abdominal testis. It may be "hernia uteri Inguinalis" or crossed testicular ectopia.
- Adults may present with malignancy of the intra abdominal gonad.
- Malignancy may also develop from the uterine segment.
- Complete excision of the Mullerian remnants carry the risk to the vas which runs along the wall of the uterus,
- Orchiopexy is more often done by splitting the Mullerian structures in the midline and removing the mucosa to prevent later malignancy.

References

1) Fernandes ET, Hollabaugh RS, Young JA, Wilroy SR, Schriock EA ("Persistent müllerian duct syndrome". primary. Urology. 36 (6): 516–8. (December 1990).

2) Rajan Garg, Jayant Radhakrisnan Minu Bajpai: Persistent Mullerian duct syndrome, Progress in Paediatric urology Volume 13. page 109-115.

3) Gujar NN, Choudhari RK, Choudhari GR, Bagali NM, Mane HS, Awati JS, et al. Male form of persistent Mullerian duct syndrome type I (hernia uteri

inguinalis) presenting as an obstructed inguinal hernia: A case report. J Med Case Rep 2011;5:586.

4) Patil V, Muktinaini S, Patil R, Verma A. Persistent müllerian duct syndrome: A case report. Indian J Surg 2013;75 Suppl 1:460-2.

5) Picard JY1, Cate RL, Racine C, Josso N: The Persistent Müllerian Duct Syndrome: An Update Based Upon a Personal Experience of 157Cases. Sex Dev. 2017;11(3):109-125.

6) Clemente A, Macchi V, Berretta M, Morra A. Female form of persistent müllerian duct syndrome: MDCT findings. Clin Imaging. 2008;32:314–7.

7) Bhatnagar KK: Uterus presenting in an inguinal hernia of a male subject. Br Med J 2:1236 (1962).

8) Binns JH, Cross RM: Hernia uteri inguinalis in a male. Br J Surg 54:571-575 (1967).

9) El-Gohary MA: Laparoscopic management of persistent müllerian duct syndrome. Pediatr Surg Int 19:533-536 (2003).

10) Gricourt S, Treton D, Renard-Pennat R, Samuel LJ, Bitker MO, et al: Novel Anti-Mullerian Hormone (AMH) mutation revealed by haematospermia in a 60 year's old patient. Clin Endocrinol (Oxf) 74:404-405 (2010).

11) Picard JY Cate R.L., Racine C. Josso N. The Persistent Müllerian Duct Syndrome: An Update Based Upon a Personal Experience of 157 Cases; Sex Dev.

12) Thiel DD, Erhard MJ: Uterine adenosarcoma is a boy with persistent Mullerian duct syndrome: first reported case. J Pediatr Surg 40:e29-e31 (2005). 2017;11:109-125 Review article.

13) Kovachev SM, Nikolov SD, Mihova AP: Uterine leiomyoma in a man with persistent Mullerian duct syndrome and seminoma. Isr Med Assoc J 16:735-737 (2014).

14) Farag TI: Familial persistent Müllerian duct syndrome in Kuwait and neighbouring populations. Amer J Med Genet 47:432-434 (1993).

Chapter 9

Approach to the Parents with a DSD Child

The initial contact with the parents of a child with a DSD with the team of health care workers is important.

In the context of a DSD diagnosis, the information conveyed to parents in the first hours after the birth of the infant will be imprinted on their minds for years to come. This is a psychological emergency and the consultant should be available to reassure the parents instead of the junior duty doctor to disclose the fact. It should be explained to the parents that though the rearing sex is not initially clear, the health care team will work with the family to reach the best possible decision in the shortest time possible. It should be emphasized while privacy needs to be respected, DSD is not shameful.

The condition can be explained to the parents as naturally occurring variation and avoiding terminologies or observations which might hurt the sentiments of the parents or the older children. The team looking after the child should avoid emotionally driven decisions, delay non-urgent decisions (such as those on surgery) until psychological counselling has been given and promote the participation of trained peers in the decision-making process. It is crucial that patient follow up continues throughout their lives in dedicated reference centers, where possible concerns still exist.

The goal of DSD treatment is the long-term physical, psychological, and sexual well-being of the patient. Controversy exists around the

ethics of performing genital surgery between individuals, families, professionals, ethicists, and activists in the treatment and management of DSD conditions. Social and health activists want decisions on treatment to be delayed until the children reach the age of consent. Parents often do not agree with this.[1]

Parents and Family Members

The health care team should discuss with the parents what information to share in the early stages with family members and friends. Parents need to be informed about sexual development and web - based information may be helpful, provided the content and focus of the information is balanced and sound. Anecdotal case reports in the form of photographs and videos may help. Long term follows up of case reports who are married and have normal sexual life gives immense confidence to parents.

Since it is impossible to know the mental inclination of neonates and infants, informed decision of parents should prevail. Prolonged uncertainty of gender is better avoided and early sex assignment is recommended although it may be far from ideals.[2] Though genital surgery can be defered assignment of sex is imperative in Indian context.

Parental Education

It becomes very important to explain patiently without any ambiguity the various aspects of biological indices of sexual development like karyotype, the functions of gonads, hormone production genital type and the immediate issues and the long-term plan. The main problem that the parents may face is disclosure of the child's DSD to extended family and friends. It is important to guide the parents when and what to disclose to the immediate relatives and friends.

Timing of Disclosure

Complete disclosure of all the facts of the child should be done in the initial stage itself otherwise the parents are likely to make their own observations based on the knowledge obtained from the internet. The choice of disclosure to the child depends on the age of the child and the diagnosis of DSD. Older children who can understand the complexities of DSD must be included in decision making, if gender change is contemplated.

In younger children, it is the parents who communicate with the child at an appropriate time based on the emotional and psychological aspect of the child. Some parents totally skip the information to the child and may lead to a great psychological problem in the child.

In a study done on adopted children and donor conceptions of children, 43 % of parents did not want the disclosure to the child.

Parent Centered or Patient Centered

What has been observed during consultations is more parent-centered care rather than infant-centered care, more an attempt to share decisions than letting the long-term interests of the child prevail. In their discussions of the pros and cons of surgery, parents and clinicians mainly orient to their concerns about the child's current medicalized predicament rather than to a future where the child can participate in decisions.

Long Term Quality of Life

The parents would like to know even in infancy the quality of later life whether the child can have normal sexual activity, the opportunity to marry, and to raise children, regardless of biological indicators of sex.

Parental concern about Fertility

Most parents identified fertility as a key concern, both at the time of

diagnosis and throughout development. Parents are dissatisfied with potential infertility to their children at the time of disclosure of long-term results. (Leydig cell hypoplasia, Mixed gonadal dysgenesis Ovo testicular DSD, CAIS, DMP etc.). However, the possibility of fertility preservation in CAH and ovotesticular DSD gives confidence in the above conditions to the parents.

Parents and External Appearance of the Child

Pressures are exerted on the surgeon for the early reconstruction of the external genitalia to make the child look normal. It needs to be explained to them that appearances during childhood, while not typical of other children, maybe of less importance than functionality and post pubertal erotic sensitivity of the genitalia. Surgery can potentially impair sexual/erotic function and can be done at an appropriate time.

Undecided and Dissatisfied Parent

There are a group of parents who cannot make a decision regarding the rearing sex inspite of a detailed explanation of the disorder. All you can do is to hear patiently his views and give all the information and guide him to some reading.

The team and the surgeon should give all the information they need and then ask the parents to determine what is in the best interest of the baby and family.

Concerns exist with regards to the effects of delayed genital and gonadal surgery on social acceptance, psychological well-being, parent-child bonding, body image and sexual functioning as well as the malignancy risk of retained gonads.

Conclusion

In India where prenatal sex determination is illegal, the birth of a child with the ambiguity of genitalia produces immense emotional and

psychological stress on parents. Guiding them to arrive at the appropriate sex of rearing of the child is in the hands of health care workers. The interaction that is necessary between the caregiver and the parents need to be patient and honest taking into consideration the long-term interest of the child especially on the functional aspect rather than the cosmetic aspect.

References

1) Minu Bajpai. Disorders of Sex Development: The quintessence of perennial controversies. J Indian Assoc Pediatr Surg. 2014 Jan-Mar; 19(1): 3–4.

2) V.Raveenthiran Neonatal Sex Assignment in Disorders of Sex Development: A Philosophical Introspection. J Neonatal Surg. 2017 Jul-Sep; 6(3): 58.